Investigations into the Mechanism of Eruption of Teeth

by

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CONTENTS.

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EARLY THEORIES ON ERUPTION.

As is the case in so many physiological matters, the first clear recognition of the existence of a problem in regard to the mechanism of eruption of teeth is to be found in the writings of JOHN HUNTER (1778).

Hunter thought that the growth of the root, or fang, as it was then called, was responsible for the eruption of the teeth. "When the fangs form, they push up the bodies of the teeth, through the sockets, which waste and afterwards through the Gum, which also wastes". (op.cit.)

He amplified his beliefs on the subject by explaining that the gum was not cut by simple pressure, but by inflammation and subsequent wasting. He stated that the cause of the inflammation was the tooth itself which was an "extraneous body" and, as such, caused irritation in the same way as pus in an abscess or a bony sequestrum.

He suggested that the eruption of a tooth was brought about by the same process as the extrusion of a foreign body or piece of dead bone; that is, by the production of inflammation and subsequent wasting of the parts between the body and that part of the skin nearest to it.

He/

He thought that this explanation accounted for normal eruption and also for teething troubles in infants.

Since the eruption of teeth is by no means invariably accompanied by inflammation, Hunter's ideas on this subject were soon forgotten. This inflammation, when it does occur, is generally assumed to be due either to a stagnation of food and tissue debris around the crown of the erupting tooth, or to a traumatic effect of chewing on the soft tissues overlying the crown of an erupting tooth.

The explanation of eruption which was generally held throughout the nineteenth century was first succinctly stated by THOMAS BELL (1835) in his book "Anatomy, Physiology and Diseases of the Teeth".

Bell was lecturer on anatomy and diseases of the teeth at Guy's Hospital and Professor of Zoology in King's College, London.

Bell stated (op.cit., p.65): "As ossification proceeds, the roots of the teeth continue to elongate, until first those of the incisors, and subsequently the others, can no longer be contained within the alveoli, and preparation is made to facilitate their passage through the gums by absorption of the containing parts. When the tooth has arrived at this stage, it presses upon the gum, a portion of the sac being still interposed; and, as this membrane has already secreted the enamel, it becomes absorbed at the point where pressure is first made, and the gradual removal of the sac and gum is the consequence".

For/

For the next hundred years this theory was repeated in all the standard texts on dentistry and anatomy, for instance in all the editions of TOMES' "Dental Surgery".

However it did not satisfactorily explain two fairly frequent occurrences which most clinicians must have encountered. These were the occasional eruption of teeth with fully formed roots, usually at a period much later than their average eruption time; and the eruption of teeth in which practically no root had formed, in this case at a period some years earlier than the average eruption date.

Because of this difficulty, from time to time alternative theories of eruption were put forward, but these were, in the main, speculative and none was based on experiment or on more accurate observation of the process of eruption than had been made previously.

COLEMAN (1881) suggested that teeth erupted by reason of their being carried through the bone by "bone currents". This theory, as put forward by COLEMAN, necessitated the existence of a process of bone growth by interstitial expansion, and as John Hunter's experiments had shown that bone growth was primarily by surface deposition, Coleman's theories were received with little enthusiasm.

However, Coleman's views foreshadow those of BRASH and SICHER to a remarkable degree, although neither BRASH nor SICHER accepted interstitial bone growth. It is rather surprising that references to COLEMAN as the original proponent of the bone growth theory of eruption are seldom found.

Similarly/

Similarly, PEIRCE (1887) suggested that proliferation of the pulp tissue might provide the propulsive force required to cause the teeth to erupt into the mouth and this theory also is now widely held.

In regard to patients with delayed eruption and cases where a tooth has lost its antagonist and "over-erupted", PEIRCE had a novel explanation. "The repeated closing of the jaws must exert, to a large extent, a mechanical force, just as the bung in a barrel is elevated by a blow being struck upon the stave on either side of it".

The most intriguing theory suggested was that by DELABARRE. It is an example of an "analogy theory", which were popular at the beginning of the nineteenth century. This popularity was largely the result of the widening knowledge of embryology which was bringing forward evidence showing similarities, or supposed similarities, in development, between widely different end-products.

Delabarre's theory was quoted and supported in a number of text-books including "The Principles and Practice of Dental Surgery" by Chapin A. HARRIS (1863), from which the following account is taken: "The able physiologist and learned dentist, Delabarre, has advanced a most ingenious theory upon this subject. He believes that the passage of a tooth through the gum, or rather its escape from its crypt, is effected in precisely the same manner as in the birth of a child. He regards the sac, attached above to the gum and below to the neck of the tooth, as the chief agent in the eruption, and believes that it is by its contraction that the latter is raised from the bottom of the alveolus/

alveolus, and ultimately forced through the dilated orifice of the capsule and gum".

This theory did not long survive the general usage of the microscope, as, of course, no muscle is present in the area.

In 1900, CONSTANT put forward a theory to explain tooth eruption which is still extant in practically unchanged form. In fact, it has received more support in recent years than any other hypothesis, having been supported by BRYER (1957), STURMAN (1957) and NESS (1959). In view of this continued support, CONSTANT'S paper merits some consideration.

It is a well written and closely argued article. The reasoning is based on observations of dissected specimens and reference to histological studies made by others - chiefly CHARLES TOMES.

In describing the anatomy of teeth prior to and during eruption, Constant emphasised that in all cases there is a mass of gelatinous, vascular tissue between the open apices of the roots and the bone of the crypt. He pointed out that this is the case in both deciduous and permanent teeth, and, from his own dissections, confirmed that it is also found in the pig and lamb.

Further, in the case of persistently growing teeth, he said "the anatomical relationship of such teeth are precisely similar to those of human teeth with partially developed roots".

He/

He criticised the elongation of the root theory chiefly because "it is extremely difficult to conceive such a process as dentine formation exercising independent mechanical force,"..... and later, "but, granting that it may be so, upon what structure is that force exercised - in other words, to put the matter clearly and concisely, if somewhat vulgarly, what does the root shove against"?

He answered this by stating that it must be the gelatinous vascular tissue which he had found to be universally present, arguing that this tissue must produce a reactive force and that this must come from the blood pressure in it, and further that the force could never be greater than the blood pressure as otherwise it would result in necrosis of that tissue.

He then went on to suggest that it was unnecessary to postulate any force from the root growth, as the blood pressure alone could account for the movement of the tooth.

CONSTANT then adduced the following arguments in favour of the theory:-

1) He believed that on mechanical or geometrical grounds alone, it was obvious that blood pressure could cause a movement of a tooth in the direction of the mouth, and illustrated this point diagrammatically. (Fig.1, which is a reproduction of Constant's drawing).

2) The vascularity of the periodontal membrane could account for continued eruption in unopposed teeth.

3)/

Redrawn from CONSTANT (1900)

Fig. 1.

3) The theory would account for the cases of teeth where the crown travels a greater distance than the length of its root.

4) Where unerupted teeth have normally developed roots, blood pressure acting as it does in all directions, could have made room for the developing root in the line of least resistance.

5) It would account for the continual eruption of teeth, as occurs in some species.

CONSTANT'S first argument listed above is the basis of the entire theory. It has been widely reproduced and as recently as 1957, BRYER published drawings illustrating the same principle in relation to both continuously erupting teeth and teeth of limited eruption.

Most writers on this theory have implied or stated explicitly that the extrusive pressure is derived directly from the pressure in the blood vessels which is transmitted through the soft tissue of the pulp and periodontium to the calcified tissue of the root surface and the interior of the pulp chamber.

This concept of the source of the extrusive pressure has been refined by NESS (1959) who suggested that the pressure was derived not directly from blood pressure but rather from the hydrostatic tissue pressure which is believed to be present in all tissues.

NESS pointed out that tissue pressure depends on tissue being contained in some envelope which limits the flow of interstitial fluid. He suggested that the magnitude of the pressure must be controlled by the blood and lymph flow in the areas.

The/

The concept of a hydrostatic pressure in tissues is part of the hypothesis put forward by STARLING (1909) to explain the mechanism of water balance in the body. The actual value of this tissue hydrostatic pressure is difficult to measure, and, indeed has not been measured. It is believed to vary in different regions. (BEST and TAYLOR, 1961).

In spite of the paucity of direct evidence in favour of its existence, the idea of a hydrostatic pressure in tissues is generally accepted, as a necessary part of STARLING'S hypothesis. (Landis and Gibbon, 1933; Best and Taylor, 1961).

The blood pressure theory of eruption can be criticised thus. When an erupting tooth which has penetrated the oral mucosa is considered, and, if the concept of hydrostatic tissue pressure is accepted, then obviously this pressure acting on the tooth has a resultant in the long axis of the tooth which would tend to push it further into the mouth. Indeed, once the concept of hydrostatic tissue pressure is accepted, then it can be accepted as a possible factor in producing all eruptive movements of teeth, once they have actually penetrated the mucosa.

However, it does not satisfactorily explain how teeth move towards the mouth while they are still entirely embedded in the tissues. For tissue pressure to cause the tooth to move toward the mouth in such a situation would necessitate the existence of pressure gradients within the tissues in the area. These have not been demonstrated.

Yet/

Yet another theory to account for eruption was postulated by WARWICK JAMES (1908) which he derived from a histological examination of erupting teeth.

In his paper, he described the position of the epithelial "glands" of Serres from sections of human material of from 2 weeks of age upward. The presence of epithelial debris in the gubernaculum suggested to him that there is always complete epithelial continuity between the enamel epithelium and the oral mucosa. Apparently he was unaware that this epithelial debris was the same as that described by SERRES (1817) as mucous glandular tissue but this point was brought out in the discussion following the paper by HOPEWELL-SMITH.

WARWICK JAMES then described sections showing cystic proliferation of this epithelial debris which he named epithelial coils. These epithelial coils, he suggested, were a means by which degeneration of the overlying tissues was produced, and in degenerating themselves, they caused the degeneration of the surrounding connective tissue. The result of this was that a pathway was opened up through which the tooth moved.

This conception was destroyed by the work of BRASH (1924, 1928) who showed that, rather than degeneration of the tissues occurring overlying erupting teeth, these tissues were actively growing toward the occlusal plane. The epithelial debris which WARWICK JAMES described is now considered to be remnants of the dental lamina. Some of these remnants do proliferate and produce small cysts containing keratin, or they may contain fluid material in which case they can enlarge to become clinically significant/

significant, as one type of eruption cyst.

Finally, one other theory of eruption may be mentioned briefly. It was put forward by SHIBATA (1929) who claimed to have ioslated a substance from enamel organ cells and cells of the dentine papilla in the rat, and suggested that this substance was a hormone which controlled eruption. However, his experimental work is unrepeatable as insufficient description of his method was published, and he published no figures on rates of eruption in rats nor described his method of measurement of this rate.

NESS (1956) carried out an experiment to establish the existence of such a hormone, but could find no evidence in favour of its existence.

TEETH OF PERSISTENT ERUPTION.

Since most of the research done in recent years into the mechanism of eruption of teeth has been carried out on the continuously growing rodent incisor, it is necessary to consider the basic assumption made by most workers that the eruption of these continuously growing teeth is essentially the same process as the eruption of teeth of limited growth.

Ness (1960) suggested that in animals where a great deal of grinding of food was necessary for adequate nutrition, two evolutionary possibilities were possible to obviate curtailment of life by premature loss of teeth. One of these was to replace the worn tooth by a successor and there are numerous examples of this phenomenon in all phyla of the animal kingdom. In mammals, this is found in elephants, manatees, dugongs and wart-hogs in relation to the cheek teeth. The other possibility is that the worn tooth should continue to grow throughout the life-span of the animal and it is with this type that we are presently concerned.

All theories of the evolution of mammals hold that, in the primitive mammal, the teeth were of limited growth and their shape was completed during the period of growth of the bearer (Scott & Symon, 1961; Ness, 1956). It must be assumed, therefore, that teeth of continuous/

continuous eruption have evolved from teeth of limited growth. There is no evolutionary evidence of the reverse transition.

As Ness (1956) pointed out, continuous eruption has appeared in widely divergent orders, and this suggests that it is based on the exploitation of processes already present in teeth of limited growth.

As the presence of both continuously erupting teeth and teeth of limited growth is found in one animal, for example, the rat or the aye-aye, it can be concluded that the change to continuous eruption is not the result of some change in a central controlling process.

Intermediate forms of teeth, from the point of view of their eruption, are found in many herbivores,for example in the artiodactyla and perissodactyla.

In these animals the premolars and molars continue to erupt for most of the life-span of the animal, but eventually, distinct roots form, and the eruption ceases soon afterwards.

These teeth link up the eruption of teeth of limited growth as found in man, for instance, and teeth which erupt continuously throughout life.

It seems reasonable, then, to deduce that information may be gained from investigations of the mechanism of continuous eruption which would be relevant to the mechanism of eruption of teeth of limited growth.

Ness/

Ness (1960) listed the occurrence of teeth of continuous eruption in mammalian orders. Cheek teeth of continuous eruption are found in some marsupials (wombats), in lagomorphs and in some rodents, for example, the guinea-pig. Tusks are found in pigs, deer, elephants, dugongs, walrus, narwhals and hippopotami.

Continuously growing incisors occur in all the order Rodentia and in the order Lagomorpha. They are also found in the wombat (marsupial), hydrax and in the permanent dentition of one primate, the aye-aye.

The rat, rabbit, guinea-pig and mouse have all been used in investigations on eruption.

Examination of the incisor of a rat, or, more obviously of the tusk of a wart-hog, shows that these form a corkscrew spiral, and not just a curve in one plane.

Spirals of this type found in animals were discussed by D'Arcy Thompson in "On Growth and Form" (1942) and he showed that such spirals, and also those formed by claws, beaks and horns, were equiangular or logarithmic spirals. In the case of continuously erupting teeth, the pole of the spiral lies at the tip of the first formed enamel which is soon lost by attrition. Therefore a continuously erupting incisor, at any one point in time, is part of an equiangular spiral, the pole of which is no longer apparent.

Methods of Measurement of the Rate of Eruption in Continuously Growing Incisors.

Oudet (1823) was the first person to prove the phenomenon of persistent eruption by cutting off rat incisor teeth at the gingival margin, and observing that the teeth were regenerated.

MacGillavry (1875) first measured the rate of eruption by making a mark on the labial enamel, measuring the distance from mark to incisal edge, and repeating the measurements a few days later. (Fig.2)

This method has been used by many investigators since (Addison & Appleton, 1915; King, 1937). The mark is usually made with a fine file or by a carborundum disc mounted on a dental engine. The measurements are made either by calipers or by direct observation of the incisors through a microscope with a calibrated graticule in the eyepiece, and requires general anaesthesia.

The objection to this method of measurement is that it assumes a constant length for the exposed portion of the incisor. This assumption is obviously not warranted as variations in the rate of attrition will result in an altered length of exposed.incisor. In other words, the measurements made by this method are the resultant of rate of eruption : rate of attrition.

To/

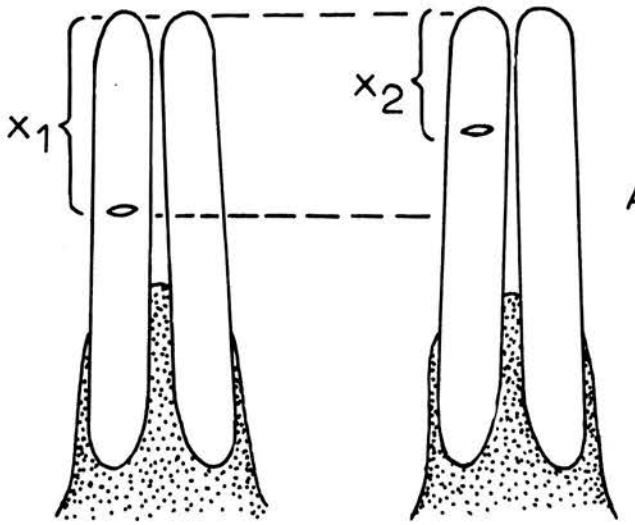


Fig. 2

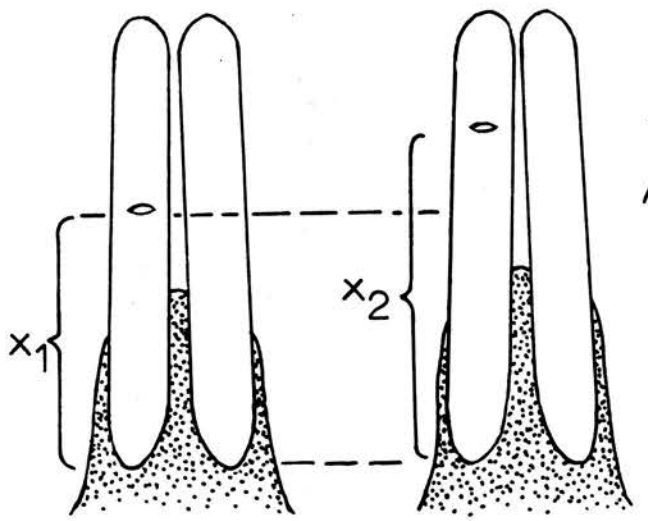


Fig. 3.

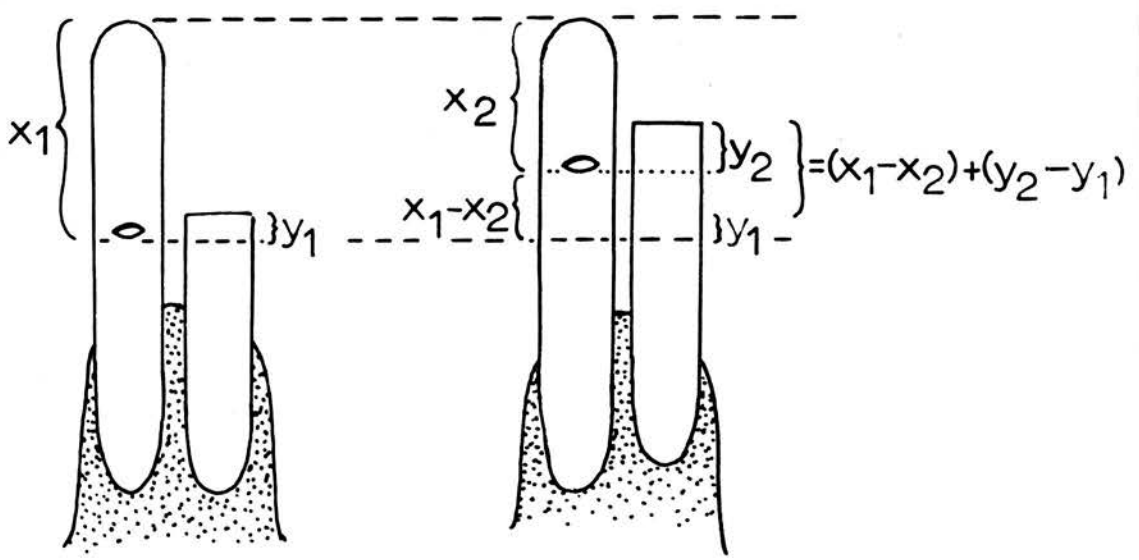


Fig. 4.

To overcome this objection, Schour & van Dyke (1931) introduced a method of measurement from a mark on the labial enamel to the highest point of the gingival crest. They made their measurements with an adjustable caliper with fine points, the distance between the points being measured subsequently on a stage micrometer in twentieths of a millimetre.

Schour & van Dyke were the first authors to subject their results to statistical analysis, publishing their results in means with standard deviations. They do not, however, give an estimate of their error of method.

Objections to this method have been made on the grounds of the variability of the position of the free edge of the gingivae, as rates of eruption are usually given in microns per day. It is unlikely that the position of the free edge of the gingivae is stable enough for measurements to this degree of accuracy to be made, especially as the experimental variables may have an effect on the soft tissues, either directly or indirectly, by affecting masticatory function or apetite. Also it must be difficult to ensure that the same spot on the gingivae is used every time, as the free edge is curved.

However, this method has been widely used (Taylor & Butcher, 1951; Baume et alia, 1954a, b & c; Garren, 1955; Sturman, 1957; Sobkowski, 1959).

Dalldorf/

Dalldorf & Zall (1930) described a method of measuring eruption rate in guinea pigs which was as follows. Using a pocket finger nail clip, the exposed portion of one lower incisor was removed every fifth day. If the tooth was too brittle, a cut was made initially with a carborundum disc on a dental engine, and the tooth then clipped. This manoeuvre could be carried out without anaesthesia. The length of the clipped fragment was then measured with vernier calipers.

Their results are given as mean eruption rate in millimetres per day, with the standard error of each mean. The means are of the order of 0.300 to 0.850 m.m. and are given to three decimal places. The standard errors range from 0.0002 m.m. to .003 m.m.. The maximum number of animals used in any one group is 10 and the number of measurements in this group 4.

In view of the findings of other workers and the crudity of the method of measurement, such small variations in eruption rate as these are incredible.

Miller (1957) measured eruption rate in the rat mandibular incisor by marking the labial enamel with a revolving diamond instrument, and taking straight lateral radiographs of the skull, under ether anaesthesia, at 7 day intervals.

He measured the distance from the mark to the most anterior part of the alveolar crest of the incisor.

It/

It would appear that this method of measurement has the possibility of being fairly accurate, if a suitable head holding device could be devised so that the position of the head was standardised. Miller does not describe any such device. Neither does he give a statistical analysis of his figures. His results are expressed in weekly eruption rates in millimetres, presumably mean values, but no standard deviations are given.

Bryer (1957) introduced a new concept into experiments on eruption rate in rats, in that he measured the eruption rate of incisors which had been cut back out of occlusion. He argued that this removed the attritional factor in eruption rate which was an unknown quantity and dependent on a number of uncontrollable factors which he enumerated as:-

1. Functional attrition, which is related to masticatory activity and varies with the quantity and consistency of the diet.
2. Gnawing of hard objects such as the metal bars of cages, etc..
3. Special attritional activity; the grinding or scraping of upper and lower incisal tips against each other. This activity may be dependent on numerous factors such as state of health, excitability, metabolic activity, irritability, nervous demands made by the particular experiment, temperature and other environmental factors.
4. The structure and quality of the incisors.
5. The eruptive force of the incisors.

Bryer/

Bryer quoted the work of Schour & Medak (1951) and Taylor & Butcher (1951) which had shown the rate of eruption of rodent incisors cut out of occlusion to be roughly double the value under physiological conditions. Bryer suggested that this value represented a full expression of the force of eruption in contrast to the values found under normal occlusal conditions which were an expression of an attrition - eruption resultant; the resultant of the eruptive force and the inhibiting force of functional occlusion. He suggested that previous experiments, in which a change in attrition - eruption rate had been found, might result from a change in the attrition rate and not in the eruptive force.

It would seem that this is a valid argument, although it will be shown subsequently that Bryer's method of measurement of eruption rate did not circumvent this difficulty.

Bryer's method was as follows:-

One mandibular incisor of an anaesthetised rat was cut out of occlusion by some 3 or 4 m.m.. The other incisor was marked by a scalpel at approximately the level of the cut surface. The distance from mark to incisal edge was measured and the distance from cut surface to mark. The measurements were made under incident light using a binocular microscope incorporating a micrometer gauge, and this procedure was repeated every 48 hours (Fig. 4).

The/

The amount of eruption of the uncut incisor he assumed to be $x - x'$ while the amount of unimpeded eruption of the other incisor is equal to $(x - x') + (y' - y)$.

However, it is obvious that in the latter formula, variations in rate of attrition will affect the measurement $(x - x')$. This method, then, does not circumvent the difficulty in relation to attrition rate which Bryer had himself described.

Ness (1954, 1956) described a radiographic method of measuring the rate of eruption of the rabbit mandibular incisor. He inserted amalgam fillings as markers and took dental radiographs twice weekly under standard conditions.

The measurements were made from the radiographs, magnified 20 times and projected on to squared paper. The reference point, from which measurements were made, was the inter-incisal septum of bone. Ness suggested that there was less likelihood of variation in the position of the bone than in the soft tissue overlying it, which had been used previously as the point of reference by Schour & van Dyke (1931) and many others.

As anaesthetic, he used bromethol and noted that no ill effects from this were detectable after repeated use in the same animal for up to one year.

An analysis of his measurement error was made and the total standard deviation resulting from errors of method was found to be $\pm$ 0.043 m.m. for any one measurement.

It/

It should be remembered that eruption of the rodent incisor, follows a spiral course, and all methods of measurement express this as a linear measurement.

Measurement of Rate of Eruption of Teeth of Limited Growth.

The only method published for this has been by Burke & Newell (1958). They measured the rate of eruption of the maxillary incisors in a child.

The child's head was held in a cephalostat which had, as its chief component, a bite plate which fitted to the occlusal surfaces of the posterior teeth. A camera was attached to the apparatus and daily photographs of the maxillary teeth were taken. These were superimposed using the occlusal plane as the baseline, and measurements of the amount of eruption made.

Burke & Newell found that the maxillary incisor erupted 5 m.m. in about 160 days. This rate may be compared with 2 m.m. per 7 days which is the normal value for the maxillary incisor in the rat.

Anatomical/

Anatomical Studies on Erupting Teeth.

The first major scientific investigation of bone growth since John Hunter's work at the end of the eighteenth century was carried out by Brash (1924, 1928).

Although Brash's work was chiefly on bone growth and, in particular, on the growth of the skull, some of his findings are particularly relevant to the problem of the eruption of the teeth.

He used madder dye to measure the amount of bone growth. It had been known for a very long time that, if madder was fed to an animal, it stained the bones red, new bone which was formed during the period of madder feeding, being more deeply stained than previously formed bone. However the line of demarcation between this newly formed bone and the previously formed bone was not very distinct, so Brash used what he termed the indirect madder method. He fed pigs on madder from birth and then stopped the madder for a few weeks prior to killing. The result of this was to stain all the bone red with the exception of that formed subsequent to stopping the madder. This gave a clear line of demarcation between red and white bone. Brash used the pig as his experimental animal.

The/

The first general conclusion which Brash pointed was that "there is no evidence of growth of bones by interstitial deposit, although interstitial changes, by deposition and absorption undoubtedly occur". The writer can remember well Professor Brash emphasising this point in his anatomy lectures to the students of this University. This finding is of basic importance to the interpretation of bone growth, and is also of basic importance in the matter of eruption. It completely destroys Coleman's concept of bone currents wafting teeth to the surface.

He also showed clearly, for the first time, that in the growing pig, there is continual growth of the alveolar processes. This alveolar growth accounts almost entirely for the increase in height of the body of the mandible. This growth is by surface deposition of new bone.

Brash measured this bone deposition on the alveolar process from sections of macerated specimens of madder stained bone. He gives figures for individual rates of growth of each animal and also average rates of growth for the periods covered by his investigations, i.e. pigs up to 28 weeks old. Similar figures are derived from measurements of bone deposition on the lower border of the mandible, and it is from comparison of these figures that he arrives at the conclusion that "alveolar growth is mainly responsible for the increase in height of the body of the mandible". His average figures show that 90% of the increase in height is due to increase in alveolar height, 10%/

10% to deposition at the lower border. A similar comparison is not easy in the maxilla since measurements of growth of the maxillary alveolar process are more difficult to compare with measurements of bone deposition elsewhere in the maxilla, for example, at the sutures.

It now seems probable that Brash underestimated the amount of sutural growth in the skull, particularly with regard to the bodily movement downwards and forwards of the maxillary complex. This, however, in no way invalidates the importance of his clear demonstration of growth of the alveolar processes, both mandibular and maxillary by deposition on their free borders. The absolute values for maxillary alveolar growth can be measured using the indirect madder method and Brash showed that these figures were somewhat less than half of the values found for mandibular alveolar growth.

It is a matter of everyday observation that the relationship of the crowns of erupted teeth to the margin of the alveolar process changes very little under physiological conditions. This is true with regard to fully erupted teeth in the young, as in adults. Any change that does take place, is a slight degree of further eruption so that more crown is exposed, and eventually a rim of cementum at the cervix of the tooth. This further eruption is considered physiological and in primitive human races, it is balanced to some extent by attrition of the occlusal surfaces of the teeth.

As/

As Brash had shown that there is continual growth of the alveolar process towards the occlusal surface, it follows that the teeth must continue to move also in an occlusal direction to maintain their relative position to the surface of the alveolar process.

Thus a completely new aspect of eruption had been introduced.

Indirect evidence of this movement of erupted teeth had been available to clinicians previously. This was from cases of "submerged" teeth where one tooth appears to sink below the level of the occlusal plane until it may eventually lie deep to the necks of the adjacent teeth and be almost completely covered by the mucous membrane. The cause of this phenomenon has been shown to be ankylosis between the root of the tooth and the bone of the alveolus (Noyes, 1932; Skillen & Walley, 1937; Vorhies, 1952).

It is most commonly found in deciduous molar teeth and the ankylosis is presumably an accidental effect during the normal resorption of the root of these teeth. Practically invariably this resorption involves not only resorption of the root but also deposition of new bone, immediately in the resorbed area. It must be a frequent occurrence for this deposition to outstrip the resorption, and ankylosis ensues (Dixon, 1963). Such ankylosis is seldom permanent, and cases where the submergence is maintained are probably due to impaction of the submerged tooth between the adjacent teeth (Dixon).

Histological/

Histological examination of specimens of submerged teeth removed surgically have confirmed the existence of the ankylosis, although in a few cases this has not been found, as one would expect, from the explanation of the condition given above.

To revert to Brash's work, he investigated, and measured where possible, these movements of erupted and erupting teeth. He showed that the movement was not all in one plane, but varied depending on the inclination of the alveolar process where each tooth lay. He found that, in the pig, the teeth in the premolar region rose nearly vertically whereas the incisors and canines moved obliquely upwards, outwards and forwards.

Brash observed that "in general, new bone is added to the walls of the alveoli and to the interalveolar septae in amount corresponding to the growth of the edge" (page 218, op.cit.).

At this point, he explains his reasons for believing that eruption is the result of this deposition of bone in the deepest parts of the crypt. To quote in full, "whatever the cause of the rise of the teeth, and let us remember that we are speaking now of the upward movement of fully erupted teeth without additions to the length of their roots, the bone growth is found in exact correspondence with it. Since this bone growth is only part of the general growth of the jaw, it is hard to resist the conclusion that we are dealing here, as I said, with another example of maintenance of relative position by bone growth and bone absorption, and that in fact, the growth of the alveolar border, with additions to inter-/

inter-alveolar septa and the filling of the alveoli from below, is actually responsible for the movement of the teeth" (page 220, op.cit.).

In addition to this explanation of eruption, Brash further suggested that the deposition of cementum acted as a contributory factor in producing vertical movement of the teeth. He suggested that the deposition of cementum was particularly important in the vertical movement of fully formed teeth, as, for example, is found in cases where the opposing tooth has been lost.

Brash adduced further observations in support of his theory of eruption from dried specimens of the manatee (Manatus Americanus), and he returned to this animal and to a group of others with similar characteristics in a lecture to the British Society for the Study of Orthodontics in 1952 (Brash, 1953). The other animals which he considered were the dugong, wart-hog and elephant. The characteristics common to this group of animals is that all exhibit "horizontal succession" of the cheek-teeth, which means that, throughout the life of the animal, molar teeth are formed, move forward in the jaws, and in turn, are exfoliated; their place being taken by more distal molars. In each animal there is a maximum number of molars which are formed. Brash had previously shown, using the madder method, that anterior movement of cheek teeth took place in pigs also, but to a limited extent, and it is generally accepted that this movement occurs also in man (Scott & Symons, 1961).

In/

In his 1953 paper, he showed that this movement, in the animals cited above, was associated with resorption and deposition of bone in the alveoli and interdental septae. This resorption could be deduced from the lacunae observable macroscopically, and the deposition from the characteristic appearance of newly deposited bone, again macroscopically.

He concluded from this that the resorption and deposition of bone was the cause of the movement forward, and that this was analogous to the process of eruption.

Brash's eruption views have been criticised on various grounds, but chiefly because they necessitate a belief that the teeth are but inanimate objects to be moved whither the bone wills. They take no account of the attachment of the teeth to the bone by means of the periodontal membrane and Brash at no time considers the adjustments necessary in this tissue to allow for the movement of the teeth. By this omission one must conclude that he thought these easily effected and of no account. Perhaps the reason for this omission is that he worked almost exclusively on dried specimens.

Brash's theory of eruption can also be criticised as it does not explain the continued eruption of teeth, such as occurs in rodents and other animals. He was aware of this difficulty himself and wrote "a consistent theory of the movement of the teeth must not only account for the ordinary movements of ordinary teeth, but must also offer an explanation of all special cases" (page 288, op.cit.).

In/

In considering the case of teeth of continual eruption, he observed first that prior to and during the first few weeks of eruption of the rodent incisor, the open apex moves backwards from beneath the mandibular molars to a position just below the condyle, this backward movement of the apex continuing simultaneously with eruptive movements of the incisal edge, and for some time subsequent to eruption. Brash notes that he has demonstrated this movement in rats by the madder method. It had previously been demonstrated in macerated specimens by Addison & Appleton (1915).

He then observes that growth of bone in the base of the alveolus cannot be the cause of continued eruption in the adult animal, as it would lead to the alveoli becoming obliterated.

He then "hazards the speculation" that continual eruption is due to "an increase in diameter of the deeper part of the tooth, the tooth accommodates itself to the slightly tapering alveolus by moving forward, a process probably assisted by the spiral curvature" (page 287, op.cit.).

This mechanistic concept is not in conflict with Brash's views on the eruption of teeth of limited growth, as, in both, the teeth are regarded as relatively passive objects which are moved by forces produced by the surrounding tissues. His views on teeth of continual growth have been disproved by Massler & Schour (1941) and by other workers, who have all demonstrated that fracture of the continuously erupting rat incisor does not prevent the continued eruption of the incisal portion, nor does removal of the growing end of the incisor prevent its eruption (Kostlan, Thorova & Skach, 1960).

These/

These findings prove that the vis a tergo, produced from the gradual increase in diameter of the incisor, has no direct part in the process of its eruption, although a very slight increase in diameter of continuously erupting teeth does occur, as was shown by D'Arcy Thompson (1942).

With regard to Brash's observations on the teeth of the manatee, dugong, wart-hog and elephant, it would seem that, no matter the cause of the horizontally-forward movement of the cheek-teeth in these animals, the bone changes which he describes would, of necessity, have to occur. To conclude that these bone changes are the cause of the movement, in the absence of any other evidence, seems to be unjustified.

It can also be argued that, if the growth of the alveolar bone is responsible for the eruption of teeth into the mouth, then occasionally, a tooth which has been lying horizontally in the bone should erupt in a horizontal position, and similarly, teeth lying in any malpositioned manner, should occasionally erupt still in the position in which they lay in the bone, that is, broadside on. This never happens and the fact that it does not must mitigate against the original hypothesis.

SUMMARY/

SUMMARY OF BRASH'S WORK.

While the latter part of this consideration of Brash's papers has been devoted to a criticism of his theories on the eruption of the teeth, it must not be overlooked that Brash's observations on the growth of the alveolar process, and on the movements of the teeth in the bone have never been challenged.

He showed clearly and indisputably that during the period of growth of the pig:-

1. The alveolar process continued to grow by deposition of new bone at its free borders.

2, That this growth occurred in the direction of the occlusal plane and also buccally.

3. That resorption of the alveolar process took place on the lingual surface but that, concomitantly, growth was occurring toward the occlusal plane on the lingual edges of the alveoli as on the buccal.

4. The teeth maintained the position of their crowns vis a vis the free margins of the alveolar process, and that this was associated with deposition and resorption of bone in the alveoli.

5./

5. That to maintain this relationship, the teeth moved vertically, buccally and anteriorly, all these movements being concomitant with the requisite deposition and resorption of bone in the alveoli.

Until the publication of Brash's observations, a theory of eruption had only to account for the movement of a tooth from its crypt to its position in the occlusal plane. Afterwards it had to account not only for this, but also for the movements necessary to keep it in its proper relationship to the free edge of the alveolus, and to the other teeth, subsequent to its full eruption as seen clinically. In other words, eruption was now known to be a process which continued throughout the entire period of growth and development of the animal, or, more precisely, a process which continued so long as alveolar bone growth continued.

THE STABILITY OF THE POSITION OF THE SHEATH OF HERTWIG

Orban (1928) reported an investigation in which he had measured the distance from the floor of the nose to the deepest part of the associated tooth germs in a series of human embryos from 16 m.m. to 217 m.m.. He also measured the distance from the most superficial part of the tooth germ to the surface of the oral mucosa.

The illustration taken from his paper, shows the result of these measurements.

A similar series of measurements were made in macerated skulls of children from which the buccal plate of the alveolar process had been removed to reveal the apices of the teeth. He measured the distance from the floor of the nose to the most apical calcified tissue of the maxillary central incisor. The results of this investigation are shown in the illustration, which is also taken from his paper (Fig. 3).

Orban's conclusions were that:-

1. No inward growth of epithelium of the enamel organ into the connective tissue takes place, either in the earliest stages of development or during the formation of the root.

2. During the formation of the root, the epithelial sheath of Hertwig remains stationary in relation to the surrounding bone and the growth of the tooth is allowed for by the formed tissue moving in an occlusal direction.

These/

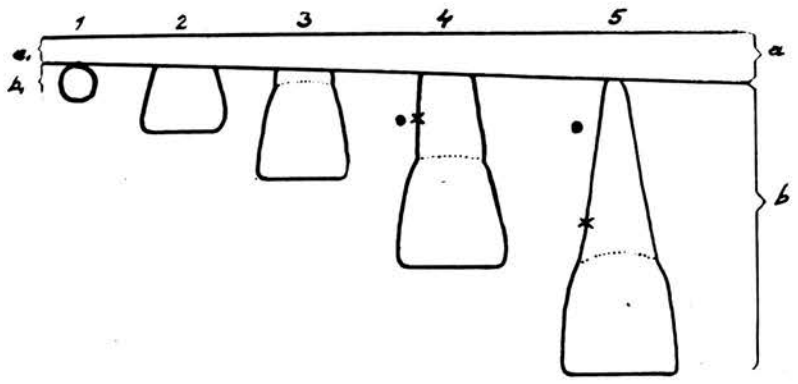


Fig. 10.—Development of the teeth and the roots. At *1*, a tooth germ is to be seen. a-a_1 is the distance from the floor of the nose to the deepest point of the teeth. At *2*, a part of the crown is already built up. At *3*, a part of the root is developed. At *4*, the root is about half grown, and at *5*, the whole tooth is already formed. The point indicated by the cross on the root in *4* and *5* moves in the direction of the mouth. The point in the bone indicated by a dot remains in its place.

Fig. 5.
From Orban (1928)

These conclusions have been disputed, and the next investigations to be described were all primarily directed to providing more information about the stability of the position of Hertwig's Sheath. Two methods of investigation have been used, cephalometric radiographs and superimposition of tracings from histological sections.

Cephalometric Investigations.

Carlson (1944) measured the rates of eruption from serial cephalometric radiographs taken at six month intervals of five children, each over a period of ten years. He orientated the radiographs by using a tangent to the lower border of the mandible as the baseline for measurement and quotes Brash's work as showing that this is reasonable, since most mandibular growth in height occurs by surface deposition on the alveolar process.

His findings were:-

1. Without exception, the distance between the tip of the crown and the lower border of the mandible increased, regardless of age, up to seventeen years of age.

2. During the period of crown formation, in the majority of cases, the distance between the forming end of the crown and the lower border of the mandible remained the same.

3. Coincident with the beginning of root formation, the distance between the forming end of the root and the lower border of the mandible decreased slightly.

4./

4. At this time also, rapid eruption of the tooth occurred, the crown tip moving rapidly away from the lower border of the mandible.

5. The rapid phase of eruption ceased when the tooth came into occlusion, subsequent to this the distance from lower border to tip of the crown increased, but slowly.

While there can be no doubt that this work is of value in showing the general trends of eruptive movements and rates in the human being, it is doubtful if the measurements can be credited with the accuracy which Carlson suggested. There is an inherent error in cephalometric radiographs due to difficulty in accurate repositioning of the head, and growth of the skull over a period of ten years leads to different film to tooth distances which also results in an inability to compare films exactly. In addition, the teeth of both sides are superimposed, and in many cases, it must be an impossible task to distinguish between the sides.

A further source of inaccuracy, which Carlson did not consider in any detail, is that there is some deposition of bone on the lower border of the mandible and also some modelling resorption. Brash found that, in the pig, 90% of the growth in height of the mandible occurred by deposition on the alveolar process, 10% by deposition on the lower border. Even if these figures also apply to man, it is still a source of considerable error.

So/

So, while accepting Carlson's main findings as likely trends, the very small movements which he describes, quoting figures of around 2 millimetres, must be considered not proven. This must apply to his finding a decrease in distance from growing root to lower border of mandible just prior to the rapid and large movement of the crown toward the occlusal plane, and his conclusion from this that the root grew into the bone at this time.

The only other investigation into tooth movements by a cephalometric technique has been that of Bjork (1955). Bjork studied the growth of the jaws in five children, aged from four to fifteen years by inserting small (0.62 m.m. x 2 m.m.) chrome-cobalt pins into the bone and taking serial cephalometric radiographs, using a standardised technique. Three or four pins were inserted into each jaw, and each child followed for two years. Bjork used the pins as reference points for superimposition of the radiographs.

It would seem that, using this method, additional information could be obtained regarding the relative movements of the teeth and the lower border and alveolar process of the mandible, at the various stages of development of the teeth.

Although Bjork implied that he had done this, he published no measurements. He did, however, publish a series of tracings made from the radiographs, with the teeth shown. These tracings show that, in the majority of cases, the developing end of the tooth bears a constant relationship to the lower border of the mandible for the period of Bjork's/

Bjork's investigation, i.e. 2 years. This relationship remains constant during the period of development and calcification of the crown and also during the period of active eruption of the tooth.

The only exceptions to this statements, in Bjork's series, are found in relation to the lateral incisor in case 1, the canine in case 11 and the canine in case V. In these three teeth, the apex of the tooth has moved away from the lower border of the mandible.

This movement has occurred in each case during the period of active eruption of the tooth.

In not one tooth in Bjork's published series did any decrease of distance from lower border of the mandible to the developing apex occur.

Histological Investigations.

Dienstein (1956) reported a series of measurements of histological sections of 10 rat mandibles from animals ranging from new-born to 31 days. Among other things, he measured the distance from the apex of the first mandibular molar to the lower border of the mandible. He found that this distance gradually increased.

The difficulties of such a method of measurement were recognised by the author. Chiefly these are the impossibility of accurately orientating the sections in the same plane to enable figures to be exactly comparable. In view of this, no great certainty can be felt with regard to his conclusions. Furthermore, the assumption that growth of the body of/

of the mandible in height is chiefly due to surface deposition on the alveolar process, which was shown to be true in pigs by Brash, and is generally held with regard to most mammals, may not be true in rodents. The presence of the continuously erupting incisor, close to the lower border of the mandible, may affect mandibular growth, and in the absence of evidence, on this matter no assumptions are justified.

O'Brien, Bhaskar & Brodie (1958) reported an investigation into the eruptive movements of the first molar of the rat, comparing the appearances in the normal animal and in rats suffering from a recessive mutation, known as ia (incisor absent) rats. This mutation had been discovered by Greep (1941) and it had been shown that the mutation was characterised by a severe retardation or absence of bone resorption although bone apposition occurred in a more or less normal manner (Bhaskar, Weinmann, Schour & Greep, 1950).

As a result of this lack of bone resorption, there are numerous fixed points of reference in ia rats from which growth measurements can be made.

O'Brien et alia compared serial histological sections between ia and normal rats from 13 days insemination age to 5 days post-natal age. The sections used were from the first molar area and cut coronally.

Using the mandibular canal, condylar cartilage and angular cartilage as fixed points, a) tracings of successive stages of ia rats were superimposed and b) tracings of identical stages of ia and normal rats were superimposed.

Their/

Their findings were:-

1. The growth movements of normal and ia rats did not differ up to 5 days post-natal age. After this, there was a deviation from normal in the ia rats, but this was not described.

2. Prior to the beginning of root formation, the tooth germ moved upward and outward, the enamel organ maintaining a fairly constant relation to the surface of the alveolar process.

3. Simultaneously with the beginning of root formation, the tooth moves rapidly towards the oral cavity.

4. At 19 days insemination age, a folding of the cervical loop of the enamel organ occurred in ia rats. This gave rise to only a very mild distortion of the enamel organ.

This work seems to confirm Orban's belief in the stability of the position of the sheath of Hertwig, in the rat at least. To be more precise, it indicated that a very slight downgrowth of the enamel organ into the bone occurred, but that, to all intents and purposes, the epithelial diaphragm did not grow downwards.

From these findings, O'Brien et alia concluded that the movement of the tooth germ prior to root formation must be due to proliferation of the tissues of the dental follicle lifting the tooth germ toward the occlusal surface.

However,/

However, it is possible that interstitional growth of the tooth germ, both of dentine papilla and enamel organ, could account for all or part of the apparent movement of the tooth germ towards the oral cavity. Measurements of the tracings which O'Brien et alia publish show that there is an increase in the bucco-lingual diameter of the tooth germ, and it seems not unreasonable to suggest that this increase would be occurring in all dimensions.

O'Brien et alia's suggestion that the movements of the tooth germ are the result only of proliferation of the cells of the follicle would seem to be an over-simplification, although, no doubt, such proliferation must also occur.

The Cushioned Hammock Ligament.

From a histological study SICHER (1942a) described a membrane of fibrous tissue stretching across the open end of the pulp chamber in teeth of continuous eruption, the fibres of this membrane being attached to the bony walls of the alveolus. Between the membrane and the bone of the base of the alveolus, he described a much looser type of connective tissue containing many blood vessels.

In a subsequent paper (SICHER, 1942b), he described a similar membrane stretching across the open apex of single rooted, erupting teeth of limited growth. However, in the case of single-rooted teeth of limited growth, the membrane contained a number of spaces filled with a mucoid-like material which/

which SICHER was unable to identify. He suggested that it was probably a type of mucoid connective tissue. Again he stated that, at the periphery, the membrane had a strong attachment to the bony, vertical walls of the alveolus. He called this membrane the cushioned hammock ligament. As in teeth of continual eruption, there was an area of much looser connective tissue between the cushioned hammock ligaments and the bone of the alveolar fundus.

In addition to this description of the periodontal tissues, he described and illustrated the formation of new bony trabeculae in the base of the alveolus of erupting teeth, that is, appositional growth of bone was occurring in this area, as had been described previously by BRASH (1928). SICHER's illustrations were from human specimens.

SICHER ascribed great significance to this cushioned hammock ligament. He suggested that, in teeth of continual eruption, it transmitted the force produced by the proliferating pulp cells to the bony walls of the alveolus as a tensile force. He argued that bone is very sensitive to any increase in pressure in the surrounding tissue, but that fibrous tissue, in this case the hammock ligament, is much better adapted to resist pressure, and that this ligament prevents the resorption of bone in the fundus of the alveolus, the increase in tissue pressure produced by the proliferating pulp cells being transmitted as tension to the bone of the wall of the alveolus.

SICHER stated that a cushioned hammock ligament was found under the open apices of both incisors of continual eruption and under the apices of cheek/

cheek teeth of continual eruption and that this explanation of eruption held for these teeth also. He stated that his histological investigations had covered the molars of rabbits and guinea pigs, but published no illustration of these.

In the case of teeth of limited eruption, he suggested that there were two sources of pressure which together caused the eruption of the teeth. The first of these was the pressure produced by the proliferation of pulp cells which was transmitted by means of the cushioned hammock ligament, just as in the case of teeth of continual eruption.

The second source of pressure was the growth of bone in the fundus of the alveolus. He suggested that the growth of these trabeculae produced uneven pressure on the open apex of the tooth, but that this pressure was equalised by means of the fluid filled spaces in the cushioned hammock ligament. He suggested that the proliferation of osteogenic tissue, rather than the actual formation of bone itself, was the source of the pressure.

With regard to multi-rooted teeth of limited growth, a different explanation of eruption was suggested by SICHER. Under the open apices of these teeth, no cushioned hammock ligament was present, but new bone formation occurred very rapidly under the bifurcation of the roots. He showed a photomicrograph of this occurring under a human second molar. He suggested that in these teeth, eruption was the result of the pressure produced by the proliferation of osteogenic tissue under the bifurcation of/

of the root, lifting the tooth bodily from its alveolus. Continued growth of the roots kept the open apices of the pulps at the fundi of their respective alveoli.

This description of the tissues lying in relation to erupting teeth has been widely quoted in the literature since SICHER's original papers were published.

However no other investigations have been published into this matter with two exceptions. SCOTT (1953) published an article entitled "How teeth erupt". The answer to this rhetorical question came under two headings:-

1. By means of the gubernaculum. This is a band of fibrous connective tissue which runs from the developing tooth follicle to the lamina propria of the overlying mucous membrane. It had been described by anatomists in the nineteenth century (TOMES, 1859) and is most easily seen in dissections of fresh specimens. Each deciduous tooth and each successional permanent tooth has its own gubernaculum. The permanent molars are all united to a common molar gubernacular cord. Epithelial debris is present in the gubernaculum. These cells are remnants of the dental lamina, and are often loosely described as the epithelial debris of SERRES.

SCOTT was of the opinion that, during the development and calcification of the crown of the teeth, it was kept in the same relation to the alveolar mucosa, which was being heightened by bone deposition on the surface of the alveolar process, by means of the gubernaculum. He envisaged/

envisaged the gubernaculum pulling the follicle containing the developing tooth upwards, in direct correlation with the vertical growth of the alveolar bone.

2. By means of the hammock ligament. SCOTT fully endorsed SICHER's description of the hammock ligament and his explanation of its function. He extended its occurrence to molars of limited eruption also and stated that in multirooted teeth, a hammock ligament developed under each root.

SCOTT illustrated his paper with diagrams but published photomicrographs of neither the gubernaculum nor the hammock ligament. He explained this omission by stating that the fibrous tissue of the tooth follicle and gubernaculum is not readily recognised in histological sections, being much more clearly seen in dissections.

It seems that this omission to publish convincing photographs of the structures to which so much significance is attached, constitutes a serious defect, and must weaken the hypothesis of the mechanism of eruption being put forward.

ECCLES (1961) reported a histological investigation which he had carried out into the basal part of the dental follicle in the rat, dog, cat, guinea pig and rabbit. Routine histological methods were used and the sections were stained with haematoxylin and eosin, MALLORY's connective tissue stain and by GOMORI's silver impregnation tecnique.

His findings were essentially the same in all the teeth which he examined, including continuously erupting teeth.

ECCLES/

ECCLES described three zones of connective tissue, viz.:-

1. an outer layer of loose connective tissue next to the bone containing a vascular plexus. This contained some collagen fibres running parallel to the bone of crypt.

2. a middle zone of less dense fibrous tissue with many tissue fluid spaces. Through it ran the vessels to the pulp and periodontal membrane. In continuously erupting teeth, reticulin fibres run axially from the pulp through the middle zone to the bone, with weak attachment to it.

3. an inner zone, not invariably present, lying close to the pulp and the epithelial diaphragm (HERTWIG's sheath and the enamel organ). This zone consisted of a thin sheet of fibres and only in peripheral sections did it appear to extend right across the basal end of the pulp. In the central axial sections, the fibres of this sheet did not run across the pulp, but turned into it in the middle of the basal area.

ECCLES also dissected some specimens and, in relation to the inner zone which he described histologically, he stated that this was attached to the root of the tooth, and not to the bony walls of the alveolus.

ECCLES concluded from this study that it was difficult to accept that the inner zone could act as a hammock ligament.

A possible criticism of ECCLES' work is that he does not state that the teeth which he examined were actively erupting, and some of his illustrations are clearly from teeth at a very early stage of development, some/

some time before active eruption begins. This criticism does not apply to the teeth of continuous eruption which he examined.

However, since SICHER (1942b) maintained that the hammock ligament developed just before active eruption commenced, some criterion of active eruption of the tooth to be examined is necessary, before any significance can be given to the absence of the hammock ligament.

NESS and SMALE (1959) noted that they could find no structure resembling the hammock ligament in the rabbit mandibular incisor.

HUNT (1959), in an extremely detailed study of the continuously erupting molar teeth of the guinea-pig, found no hammock ligament in relation to these teeth.

Tissue overlying erupting teeth.

ENGEL (1951) investigated the changes which took place in the connective tissue overlying teeth which were erupting. He studied tissue which had been surgically removed from children and also tissue from young pigs and rats.

The tissues were fixed by the freeze-drying method and stained by the periodic-acid-Schiff tecnique. In some experimental animals, Evans blue was injected intravitally.

ENGEL found that the connective tissue overlying erupting teeth stained more deeply with the periodic-acid-Schiff stain than did the same tissue during the period of development and calcification of the crown. This increase in colour was removed by treating the section with hyaluronidase prior to staining.

During/

During the period of eruption, this tissue bound larger amounts of Evan's blue than previously.

He also found that the fibroblasts of this tissue contained more glycogen during the eruptive period than before.

From these findings, ENGEL deduced that there was a depolymerisation of the muco-polysaccharide ground substance of the connective tissue overlying the tooth during the period of active eruption and that hyaluronic acid was one of the principle muco-polysaccharides present in this tissue. He did not speculate on the cause of the depolymerisation, and more work is needed to elucidate the nature of the changes in this tissue.

The Periodontal Membrane.

In relation to the continuously erupting teeth, SICHER (1942a) described three layers in the periodontal membrane on the side of the tooth where cementum was present (Fig. 13). The outer layer consisted of comparatively strong fibres coming from the bone of the socket wall toward the tooth, the orientation of the fibroblasts being similar to that of the fibres. These fibres run into an "intermediate plexus" whose fine fibres form a filmy meshwork. By their staining reactions, SICHER suggested that the fibres in this area were precollagenous. The long axes of the cell nuclei lie chiefly parallel to the long axis of the tooth. In the third layer, nearest to the cementum, strong fibres run from the intermediate plexus to the cementum.

SICHER/

SICHER suggested that the adjustments necessary to allow the tooth to move out of its socket took place in the intermediate layer.

Sicher's description has been confirmed by a number of workers and the same arrangement has been shown to exist in teeth of limited eruption during their period of eruption (Eccles, 1959).

NESS & SMALE (1959) investigated the distribution of mitosis in all the odontogenic tissues including the periodontal membrane of the rabbit incisor. They found that, in the inner zone of the periodontal membrane, mitoses were few, but that in the intermediate plexus the numbers of mitoses increased greatly. The numbers were highest in the part of the intermediate plexus nearest to the cementum, and progressively decreased farther from the cementum.

In the outer layer of the periodontal membrane, mitoses were less frequent than in the intermediate layer and many of them were in recognisable osteoblasts.

NESS & SMALE described an experiment in which holes were drilled labio-lingually through the alveolar bone and the incisor of the rabbit, and were filled with plugs of fibrin incorporating silver dust. Subsequent observations of the lines of particles showed that the tooth had moved past the bone.

They point out that this proves that there is a gradient of velocity across the soft tissue between the bone and the incisor surface.

They/

They then speculated on the explanation of their findings of the distribution of mitoses across the periodontal membrane. They suggested that the gradient of cell division across the intermediate plexus is a manifestation of the velocity gradient.

They also described the distribution of mitoses along the long axis of the rabbit incisor. They found that in the 2 m.m. nearest the base of the incisor, the incidence of mitoses was very high, and that throughout the remaining length of the tooth the distribution was fairly even. They pointed out that the basal part of the periodontal membrane is not normally considered to move relative to the socket wall, and that it is in this area that the attachment of the connective tissue fibres to the surface of the cementum becomes established.

The relative turnover rates of sulphated muco-polysaccharide in the different areas in the periodontal membrane of the rat was investigated by ECCLES (1962). ECCLES injected sulphate labelled with S.35 intra-peritoneally, and found there was uptake of isotope throughout the periodontal membrane. This was demonstrated by means of autoradiographs, and ECCLES interpreted this as the normal turnover of sulphated muco-polysaccharide in the ground substance. He fould no increased uptake in the intermediate plexus of the rat incisor, and suggested that, whatever changes take place in this to permit continuous eruption, they do not require the formation or breakdown of sulphated muco-polysaccharide.

The/

The Pulp of the Persistently Erupting Incisor.

In the paper quoted above, NESS & SMALE (1959) also described the distribution of mitoses in the pulp of the rabbit mandibular incisor. They found that 65% of all mitoses were found in the basal millimetre. The frequency then fell rapidly, coronal to the baseline, and over 40% of mitoses coronal to the basal two millimetres were in the walls of blood vessels.

In the basal millimetre of the pulp, mitoses were infrequent in the middle of the pulp, frequent at the periphery. This is in agreement with the concept of the cells of the enamel organ and HERTWIG's sheath acting as inducers on the connective tissue cells of the pulp.

NESS & SMALE also found that the cell density was highest in the basal millimetre of the pulp and that from the second millimetre coronally it remained fairly constant. GAGAN & NESS (1960) confirmed this finding and found that in the most coronal part of the pulp the cell density increased again.

NESS & SMALE found that in teeth which had been cut out of occlusion and were therefore erupting more rapidly than usual, the basal millimetre of pulp tissue contained fewer cells, but, two millimetres from the baseline, there was a band of tissue with a higher cell density than is found in teeth erupting in function. This finding has not been explained.

From/

From the finding that the cell density of the pulp is constant for most of its length, although the volume of the pulp decreases with the apposition of dentine, GAGAN & NESS argue that cells and extracellular material must be destroyed and removed. They suggest that the finding of an increase of cell density in the apex of the pulp may result from extracellular material being removed faster than cells.

EFFECTS OF VARIATIONS IN DIET ON ERUPTION

CONSISTENCY OF DIET.

The effects of dietary consistency were investigated by TAYLOR & BUTCHER (1951) who publish a graph showing that eruption rate was higher on a hard diet (corn kernels) than on a soft diet (corn meal). They also state that the visible length of the incisor varied with the consistency of the diet, a hard diet resulting in a decrease in length when compared to the visible length when on a soft diet.

It is not possible from the data given to accept that these differences are significant.

BRYER (1957) repeated TAYLOR & BUTCHER's experiments to investigate the effects on rate of eruption of variation in the consistency of the diet. Using his method of measurement of eruption rate in rats, he could not detect any differences in rate when hard or soft diets were fed.

QUALITY OF DIET.

ORBAN (1927) described a series of experiments on rats in which the diet was varied. The diets used were basically wheat with additions of meat, milk, starch, green vegetables, cod-liver oil, fat, casein, calcium phosphate, and lemon juice, in various combinations.

A/

A graph is shown indicating different rates of eruption depending on the combination of foodstuffs given.

However, adequate data for statistical evaluation is lacking and no significance can be attached to these differences.

The method of measurement of eruption rate is not described.

DOWNES (1930) described an experiment in which he measured the rate of eruption of incisor teeth in rats fed on different diets. The diets were "normal", high fat, high carbohydrate, high protein, low salt (NaCl), phosphate free and calcium free.

He published figures which show different rates of eruption as a result of these variations.

However, he does not give any figures on numbers of rats used in each group, nor of the time on each individual diet. Neither does he give any analysis of his figures, publishing only means as millimetres per week.

As the differences between the means for each group are small, and a statistical analysis not possible on the data given, no conclusions can be drawn from this study.

BRYER (1957) investigated the effect of diet on eruption rate in rats by setting up an experiment with three groups of siblings. The first group (a) received a full diet containing all essential food factors, the second group (B) received the same diet but with the protein replaced by carbohydrate, the third group (C) were fed the full diet but only half the total quantity consumed by the control group.

The/

The effects of the diets were:-

1. Groups B and C lost weight steadily.
2. No significant difference in eruption rate between group A (control) and group B (protein deficient) was found. In group C (calorie deficient) no significant difference occurred for the first two weeks, but in the last two weeks a rise in eruption rate occurred. At the end of four weeks, the animals in group C either died or became moribund and had to be killed. At this point the rate of eruption dropped suddenly.
3. The blood pressure of the rats, measured by the indirect method of BYROM & WILSON (1938), did not differ significantly from normal with the exception of group C during the 3rd and 4th week of the experiment. During this period, the blood pressure in group C fell from a "fairly normal range" of 80 - 130 m.m. to a low range of from 50 - 94 m.m.
4. Histologically the most noticeable differences between the groups were a thinning of the dentine in group C and an increase in the tissue spaces of the pulp. BRYER ascribed this finding to famine oedema.

BRYER's conclusions were that protein deficiency exerts little effect on eruption rate, but that "oedema of the pulp and periodontal tissues reflects an increased tension in these tissues, derived from the circulation".

He/

He suggested that increased eruption rate found in group C, was the result of famine oedema, which was, in turn, a reflection of increased tissue tension and that this was consistent with the blood pressure theory of eruption.

As explained elsewhere, BRYER's method of measurement does not take into account changes in the amount or rate of attrition, and it is possible that the changes in eruption rate which he found in these starving rats, were in reality a change in attrition rate. It may seem unlikely that starving rats should have an increased attrition rate, but the possibility cannot be excluded.

MAGNESIUM

GAGNON et alia (1942) showed that a diet deficient in magnesium caused a reduction of approximately 70% in incisor eruption rates in rats. With this finding, there is an associated deceleration of dentine apposition which is more marked on the dentine lying beneath the enamel than on that covered by cementum.

The total food intake of the magnesium deficient rats was approximately that of control animals and the experimental animals did grow in size and weight although at a slower rate than controls. GAGNON et alia conclude that the severe retardation in eruption is not associated with the relatively mild retardation in body weight.

This finding remains inexplicable.

VITAMIN/

VITAMIN A.

KING (1937) showed that, in dogs deficient in vitamin A, the eruption dates were delayed. In addition hypercementosis occurred and the lamina dura of the alveolus did not form. He did not measure the effects of vitamin A deficiency on eruption rate in continuously growing teeth.

SCHOUR, SMITH & HOFFMAN (1938) reported on the effect of vitamin A deficiency in rats. They found that dentine deposition was markedly reduced from roughly 12 days after substitution of the vitamin A deficient diet. They measured the reduction in rate of dentine deposition by injecting alizarine which produces a thin red line in the dentine formed at the time of injection. By giving two injections at a few days interval, and then measuring, on ground sections, the distance between the red lines in the rat incisor, they found the daily rate of dentine apposition to be 16 microms in control animals. This rate was reduced during vitamin A deficiency, long before any cytological changes were evident. However they did not measure the effect on the rate of eruption, and rate of dentine deposition and rate of eruption do not necessarily have a high degree of correlation.

BRYER (1957) found that in rats fed on diet deficient in vitamin A from weaning, there was a great decrease in rate of eruption, so that at the age of 12 weeks, the rate was approximately 80% less than that of sibling controls.

Replacement/

Replacement therapy with vitamin A after 8 weeks deficiency, restored eruption rate to approximately normal in 4 weeks.

Histological examination of the incisors of vitamin A deficient animals confirmed the findings of SCHOUR et alia (1938) with regard to decreased dentine apposition, and BRYER also found a decrease in vascularity when compared to control animals. He did not give his criteria for assessing degrees of vascularity.

VITAMIN C.

The effect of scurvy on eruption rate was described by DALLDORF & ZALL (1930) in relation to guinea pigs.

They found that the incisor eruption rate was reduced to approximately half the normal value.

Their method of measurement of eruption rate was very crude and yet they give figures of means with standard errors which are so small as to be quite incredible. Further work is needed before these findings could be accepted.

VITAMIN D.

BRYER (1957) found that rats which had been fed on a rachitogenic diet from birth had a significantly lower eruption rate than controls fed the same diet plus vitamin D. He found that changing from an adequate diet to a Vitamin D deficient diet in adult rats did not change the rate of eruption. He also found that the addition of massive quantities of vitamin D (calciferol, up to 100,000 I.U. daily) resulted in a decline in rate of eruption.

The Influence of the Endocrine Glands on Eruption.

THYROID

The effect of thyroxine on the eruption of teeth was first described by HOSKINS (1927). She found that precocious eruption of the incisors occurred in rats. This experiment was repeated by KARNOFSKY & CRONKITE (1939) who found that the appearance of the incisor in rats occurred at 3-4 days under the influence of injected thyroxine, compared with 8-9 days in control animals.

These findings were confirmed by HERZBERG & SCHOUR (1941) and they also measured the rate of eruption in animals from the ages of 14-21 days and 21-28 days. They found that, in animals to which multiple injections of thyroxine had been given, the rate of eruption was almost double that of litter-mate controls. They also found that the first molars in the experimental animals erupted at 14 days, as compared to 19 days in control animals.

They do not describe their method of measuring eruption rate, presumably the method was that commonly used by SCHOUR, in which case a difference of 100% in eruption rate would be significant.

ZISKIN et al (1940) reported experiments in which both thyroid and parathyroid glands were removed surgically at birth. In these animals the eruption times of both incisors and molars were retarded.

SHUMER/

SHUMER & WELLS (1958) showed that L - triiodothyroacetic acid, which is a synthetic analogue of thyroxine, had a similar effect in raising unimpeded eruption rate in the rat incisor as L - thyroxine. They used BRYER's method of measurement of eruption rate. They found no significant differences in opposed eruption rate between controls and animals given these two drugs. The difficulties inherent in this method of measurement may account for this discrepancy between their findings and those of HERZBERG & SCHOUR.

PITUITARY

That this gland had a regulating or controlling action on the eruption of teeth was first suggested by KEITH (1913). His suggestion was based on a consideration of skeletal remains from cases of acromegaly, gigantism, progeria and achondroplasia.

It is now generally accepted (STONES, 1962) that in gigantism in man the eruption of the permanent teeth occurs prematurely, and conversely, in hypopituitarism of the LORAIN-LEVY type, there is retardation of the eruption of both the deciduous and permanent teeth.

The first experimental study was carried out by DOWNS (1930). He investigated the effect of repeated injections of an extract of the anterior lobe of the pituitary, which had been prepared in various ways, in mice and dogs. Inter alia, he measured the effect on the eruption rate of the mice incisors. He states that he measured the rate of eruption by marking the incisor with a fine dental disc, but he does not say from what point he made the measurement.

He/

He gives figures to support his contention that the pituitary extract, no matter which method of producing it had been employed, caused an increase in eruption rate. However, statistical analysis of his published data is not possible.

DOWNS did show that injections of the anterior lobe extract caused early eruption of the permanent teeth in dogs, as compared with litter-mate controls.

SCHOUR and van DYKE (1931 & 1932), in a very detailed and thorough study of the effects of hypophysectomy on the teeth of the albino rat, showed conclusively that the eruption rate of the incisor was markedly retarded. They further demonstrated that replacement therapy with an extract of the anterior lobe of the pituitary, increased the rate of eruption in animals which had had their pituitaries destroyed, although it never reached the normal rate of eruption. In contra-distinction to DOWNS (1930), they found no change in the rate of eruption in normal animals when injected with the pituitary extract.

In a later paper, SCHOUR & van DYKE (1934), they showed that, following hypophysectomy, the eruption of molars in rats was retarded, and if the operation had been carried out early enough, the third molar frequently did not erupt at all. In these molars with retarded eruption, the apical foramina remained wide open.

BAUME et alia in a series of papers (1954, a, b, c) reported on a number of experiments into the influence of the pituitary gland and the thyroid gland on the eruption of the rat incisor.

Their/

Their conclusions were as follows:- (1954 a)

1. Thyroidectomy reduces eruption rate by approximately fifty per cent. Concurrent growth generally, including growth in incisor size, was reduced. Differentiation of the epithelium of the enamel organ was poor and atrophy soon developed.

2. Administration of thyroxine increased the eruption rate to approximately normal levels. Their published figures showed the eruption rate subsequent to thyroxine therapy to be 82% of normal. Thyroxine also caused normal histodifferentiation of ameloblasts, but calcification remained deficient.

3. Administration of growth hormone in thyroidectomised rats resulted in increased bone growth when compared with normal controls, however, no histo-differentiation of the enamel epithelium occurred, nor was there a significant increase in eruption rate.

4. The administration of both thyroxine and growth hormone to thyroidectomised animals increased the eruption rate to 83% of normal. Again the difference of 17%, is not statistically significant, at $p = 0.05$. The histological appearances of this group of animals were indistinguishable from normal.

Subsequently/

Subsequently BAUME et alia (1954 b, c) reported on the effects of hypophysectomy at 28 days of age, again in rats. Their findings were:-

1. Hypophysectomy produces an immediate reduction in eruption rate, which becomes progressively lower with time, though it never stops entirely.

2. Apical folding of the dentine occurred in 60% of the operated animals 160 days after operation. This showed a uniform histological sequence:- (a) dysgenesis of inner enamel epithelium with resultant enamel aplasia, (b) disturbed calcification of dentine at the apex of the tooth, (c) agenesis of odontoblasts.

3. Unfolded incisors continued to erupt at a much reduced rate (approximately 25% of normal) but incisors in which folding had occurred erupted at approximately 6% of normal.

The effects of replacement therapy in these hypophysectomised rats were:-

1. Growth hormone had no effect in raising the eruption rate, and no effect on the poorly differentiated enamel epithelium.

2. Treatment with thyroxine increased eruption rate by approximately 40% and amelogenesis was much improved.

3./

3. Treatment with a combination of thyroxine and growth hormone increased eruption rate even more, although it did not quite bring it up to normal levels.

BAUME et alia also confirmed the findings of SCHOUR & van DYKE that the administration of growth hormone to normal animals did not increase eruption rate.

One unexplained finding of this group of investigators (COLLINS et alia, 1949) is that in hypophysectomised animals, treated for long periods with growth hormone, the curvature of both maxillary and mandibular incisors is increased considerably, and the pulp canal becomes almost obliterated by the deposition of dentine.

The conclusions to be drawn from this excellent work are:-

1. The growth hormone of the pituitary is not primarily involved in the control of eruption, in contra-distinction to its action on the growth of bone.

2. Removal of the pituitary produces an effect of eruption largely through the mediation of the thyroid.

3. Thyroxine stimulates eruption directly, and in its absence, eruption rate is markedly reduced.

It has been suggested (NESS, 1956) and (MEDAK, WEINREB, SICHER, WEINMANN and SCHOUR, 1952) that hypophysectomy lowers the rate at which eruption is permitted, while growth of the dental tissues at the base is comparatively unimpaired. They suggest that hypophysectomy retards the rate/

rate at which the periodontal connective tissue can be reorganised to permit eruption, and that this is the reason for the folding of the dental tissues in some hypophysectomised animals.

PARATHYROID

The influence of the parathyroid on the incisor tooth of the rat was first studied, histologically, by TOYO FU KU (1911) who described the changes produced by surgical removal of these organs. He did not, however, measure the effects on the rate of eruption. This was done by GOTTLIEB (1920). He measured the rate of eruption in three rats, in two of which the parathyroid glands had been surgically removed. He found that the rate of eruption in two experimental animals was lower than in his control animals.

In view of the small number of animals used, and as he does not appear to have used siblings, no great significance can be put on his results.

SCHOUR, CHANDLER & TWEEDY (1937) repeated GOTTLIEB's measurements of incisor eruption rate in 10 animals subsequent to surgical removal of the parathyroid glands. They do not describe their method of measurement of eruption rate. They state that, in the experimental animals, the rate of eruption of the maxillary incisors was 2 m.m. per week, and of the mandibular incisors 2.8 m.m. per week, and "similar measurements in normal animals were in the same range."

While/

While one cannot accept from this account, that there is no difference in rate of eruption following parathyroidectomy, it seems likely that any effect produced is fairly small.

ADRENAL CORTEX

MULINOS & POMERANTZ (1942) first noted the precocious eruption of the incisors in rats injected with desoxycortisone acetate from the age of 1 day.

PARMER et alia (1951) described a series of experiments in which they injected various adreno-cortical hormones and ACTH into new-born rats and noted the effects on the eruption dates of the teeth.

They found that cortisone had most effect in causing early eruption of the teeth; desoxycorticosterone and corticosterone had a similar but less marked effect, and ACTH had no effect on tooth eruption dates. Cortisone also inhibited growth generally as did ACTH, but desoxy-corticosterone and corticosterone had no effect on growth.

GARREN (1955) found that daily injections of cortisone increased eruption rates in 80 day old rats. 5 mgm. of cortisone per day produced the maximum effect, an increase of approximately 30%. He could find no histological changes to account for these effects. GARREN found no change in eruption rate in rats injected daily with desoxycortisone acetate.

SOBKOWSKI (1959) performed bilateral adrenalectomies on 9 rats, carrying out sham operation on 9 others. He found that eruption rate of the incisors of the adrenalectomised animals dropped, with a maximum decrease/

decrease of 40%, 14 days subsequent to operation. At that time (i.e 14 days post-operatively), daily injections of hydrocortisone in alcohol, 4.0 mgm. per day, was begun. This resulted in a great increase in eruption rate in both adrenalectomised and sham operated controls, both groups having an eruption rate 36% greater than control, pre-operative values, 5 days after the institution of hydrocortisone injections.

SOBKOWSKI also determined that, in normal rats, the administration of hydrocortisone parenterally caused a significant increase in the rate of eruption of the incisor teeth. The maximum rate of eruption was produced by doses of 2 mgm. per day, and was of the order of 30%. Larger doses than this did not elicit a greater increase.

SOBKOWSKI's results were confirmed by DOMM & WELLBAND (1960).

No satisfactory explanation of these effects on rate of eruption has been advanced.

GONADS

Little work has been reported on the effects, if any, on eruption, attributable to malfunction of the gonads, or on any physiological relationship between eruption and the sex hormones.

ERAUSQUIN (1942) described a case of malocclusion of the incisors of the rat which he thought was the result of oestrogen therapy. This animal/

animal, a castrated female, had had a tablet of oestrogen implanted into the dorsal musculature. Subsequently it was noted that the incisor teeth were not occluding properly and damage to the soft tissues was occurring. During a three month period, the incisors were sectioned twice to allow normal mastication, and then the animal was killed.

An extremely detailed histological examination was carried out and it was found that large unilateral exostosis had formed in the facial bones. ERAUSQUIN was of the opinion that these had caused displacement of the incisors with subsequent loss of occlusal contact, attritional wear, and overgrowth.

No direct relationship between eruption and the gonadal hormones has yet been shown.

The Influence of the Nervous System.

The influence of the nervous system on the eruption of teeth was first investigated by BREITNER & LEIST (1927).

They worked chiefly on guinea pigs and their findings were:-

1. Cauterisation of the sympathetic plexus around the carotid artery with phenol results in an increase of eruption rate in the incisor teeth. They showed that this occurred unilaterally or bilaterally, and persisted for up to 8 weeks after painting with phenol.

2. Sectioning of the pre-ganglionic sympathetic nerves in the neck, before they reached the superior cervical ganglion, resulted in an increase in eruption rate of the incisors on the operated side.

3. Removal of the superior cervical ganglion itself gave equivocal results. In two cases out of six, an increase in eruption rate resulted, but not in the other four.

4. Section of the inferior dental nerve as it enters the mandibular foramen results in an increase of eruption rate on the operated side.

They suggested that this was probably due to the inferior dental nerve carrying sympathetic fibres.

BREITNER/

BREITNER & LEIST did not describe their method of measuring eruption rate, and therefore their results must remain suspect.

This work was repeated by KING (1937). He found that section of the inferior dental nerve in rabbits resulted in increased eruption rate, and that section of the cervical sympathetic nerves between the middle and superior ganglia had the same effect. He also suggested that the increased eruption rate following section of the inferior dental nerve was due to cutting sympathetic fibres carried with this nerve.

KING describes an increased vascularity in the pulp on the operated side in animals killed and sectioned for histological examination. He suggests, however, that the decreased vascularity on the unoperated side might be due to vasoconstriction as a result of asphyxia induced by the chloroform used in killing the animals. This could not have occurred on the operated side where vasoconstrictor nerves had been cut.

EDWARDS & KITCHEN (1938) described the effects on the developing teeth of sectioning the inferior dental nerve and removing the superior cervical ganglion in kittens. Section of the inferior dental nerve alone had no consistent effects on the rate of growth of the teeth, but removal of the superior cervical ganglion resulted in an increased rate of development on the operated side when compared with the unoperated side. EDWARDS & KITCHEN investigated KING's (1937) suggestion that the effects he produced by sectioning the inferior dental nerve were due to section of sympathetic fibres in this nerve. They state that there is no evidence/

evidence to support the suggestion that sympathetic fibres are carried in the inferior dental nerve. They describe microscopic investigation in which they demonstrated the presence of amyelinated nerve fibres in the adventitia of the inferior dental artery. These fibres are post-ganglionic sympathetic nerves from the superior cervical ganglion, they state, and section of the inferior dental nerve could not affect their action.

TAYLOR & BUTCHER (1951) repeated these experiments on rats. They found that:-

1. Cutting the inferior dental nerve resulted in an acceleration of eruption rate on the operated side of approximately 20% to 30% over the previous rate. The tooth on the operated side also became shorter than the other incisor. Microscopically, no difference from normal was detectable, apart from an absence or reduction in nerve fibres.

2. Removal of the superior and middle cervical sympathetic ganglia and the sympathetic chain as far as the first rib had no effect on eruption rate.

3. Removal of the sympathetic nerves described above combined with resection of a segment of the common carotid artery and its sheath gave rise to an initial fluctuation in eruption rate and subsequently a slight reduction in rate of eruption. It is dubious whether this reduction was significant.

4./

4. Resection of the common carotid artery and its sheath alone produced exactly the same effects as when this operation was combined with resection of the sympathetic nerves.

5. Removal of the otic ganglion (on the assumption that parasympathetic fibres from this ganglion might supply the teeth or surrounding structures) had no effect on eruption rate.

6. In animals where first the sympathetic chain had been removed, then 30 days later a segment of one common carotid artery removed, then 2 months later the inferior dental nerve resected, the effects on eruption rate were:-
   (a) subsequent to sympathetic section, no effect,
   (b) subsequent to resection of the carotid, a small fluctuation in eruption rate occurred, steadied to approximately normal values,
   (c) subsequent to section of the inferior dental nerve, unilaterally, the eruption rate on the operated side rose to some 30% above its previous rate.

To investigate further the effect of the sympathetic nervous system on the tooth pulp, TAYLOR (1950) devised a method for direct observation of the living tooth pulp. In anaesthetised rats, a window was ground through enamel and dentine till only a thin layer of dentine was left covering the pulp. A drop of oil was then placed on this dentine and the pulp vessels below could be observed directly, via a microscope. When electrical/

electrical stimulation was applied to the cervical sympathetic chain, in an animal under observation, there was a reduction in the rate of blood flow. However, no change in the diameter of the arterioles could be seen. TAYLOR & BUTCHER argue that this is strong evidence against the presence of any sympathetic fibres in the pulpal arterioles.

On the basis of this evidence, and their finding that section of the sympathetic has no effect on eruption rate, they argued that the eruption rate of the rat incisor is not under autonomic control.

BRYER (1957) repeated these experiments on rats and measured the effects on eruption rate, using the method he had devised to measure unimpeded eruption rate.

His findings were that section of the inferior dental nerve produced an increase in unimpeded eruption rate and section of the superior cervical sympathetic also produced this effect. In both cases, the increase in unimpeded eruption rate was approximately 15%. In view of the inaccuracies of the method of measurement, no great weight can be put on this finding.

MILLER (1957) repeated these experiments to determine the effect on rate of eruption of section of the inferior dental nerve and of the cervical sympathetic. His findings were the same as those of TAYLOR & BUTCHER (1951). He also described the histological changes in rats and rabbits produced by these operations. No change between operated and control side could be detected subsequent to section of the inferior dental nerve./

nerve. In rabbits subjected to unilateral cervical sympathectomy, he found increased osteoclastic activity on the operated side. He also found increased vascularity in relation to the increased osteoclasis. However, the assessment of vascularity in tissue sections is rather subjective, and MILLER gives no criterion on which to base his findings.

MILLER's conclusions were that the eruption rate of the rat incisor is not sensitive to large changes in rate of blood flow in adjacent tissues, and that there is a direct relationship between osteoclastic activity and degree of vascularity.

In view of the general agreement between research workers, it can be concluded that section of the dental nerve does produce an increased rate of eruption. This is most probably the result of increased attrition due to anaesthesia of the tooth and its investing tissues.

There is noticeable disagreement on the effects of sympathetic section, but the work of TAYLOR & BUTCHER (1951) is by far the most impressive and their findings have been confirmed by MILLER (1957). It would appear most unlikely that a species difference occurred between rats and rabbits in the method by which their eruption was controlled.

The Influence of the Blood Vascular System.

TAYLOR & BUTCHER (1951) investigated the effects of section of one common carotid artery on the eruption rate of the rat incisor, in three animals. They found that this produced a fluntuation in eruption rate which lasted for 4 or 5 days, in both incisors. Subsequently the rate returned to normal values.

They also sectioned the inferior dental artery in five rats, and found no effect on eruption rate at all.

From these findings they concluded that collateral blood channels must exist which provide anastomotic connections for the area supplied by the common carotid artery. To investigate this further, they observed the effect of ligating these vessels while capillary blood flow in the incisor pulp was under direct observation. They found that occluding these vessels did not stop capillary blood flow in the pulp. The only way in which blood flow could be stopped was to exert retractive tension on the tooth itself.

BRYER (1957) investigated the effect of the administration of cobalt on the eruption rate of the rat incisor. Cobalt, when administered in small amounts to rats, produces a polycythaemia (ORTEN et alia, 1932), and BRYER produced polycythaemia in 12 rats by this method. He measured the eruption rates of this group of animals and of a group of sibling controls. He found that after 2 or 3 weeks the eruption rate in the experimental group had decreased by about 5 per cent.

BRYER/

BRYER claimed that this difference was significant, but in view of the method used, his claim must be viewed with reservations.

He suggested that the reduction in eruption rate was the result of a decreased capillary pressure, in turn the result of an increased peripheral resistance due to the polycythaemia. However, he did not measure either the capillary pressure or the arterial pressure.

MILLER (1957) ligated the common carotid artery bilaterally in rats and found no change in rate of eruption of the incisor tooth. The data which he published does not permit statistical analysis.

STURMAN (1957) investigated the effects on the rate of eruption of the rat incisor of daily injections into the masseter muscle of a vasoconstrictor (levophed bitartrate) and of a vasodilator (priscoline hydrochloride). He used Sprague-Dawley rats and measured eruption rate by the method of SCHOUR & van DYKE (1931). He had three control groups, one of which received the same volume of distilled water into the masseter, another had a needle inserted into the masseter but no fluid injected, and another group which was not injected.

His results are very curious as he found that priscoline increased the rate of eruption by approximately 20%, but so did injections of distilled water and also sham injections, when no fluid was introduced into the tissues at all. Levophed produced a smaller, but still significant rise also in rate of eruption.

STURMAN/

STURMAN suggested that the explanation of these unexpected findings might be that handling rats daily increased the rate of eruption. To test this he handled his uninjected control group (20 rats) daily for one week. During this week their rate of eruption rose to levels similar to those animals in the other groups.

This handling effect has not been reported by any other worker, and in the absence of confirmation, judgement must be withheld. STURMAN suggested that the effect might only be found in Sprague-Dawley rats.

Miscellaneous Experiments Designed to Affect Eruption.

Effect of Cutting One Incisor Back Out of Occlusion.

SCHOUR & MEDAK (1951) described the effects of cutting off (twice weekly) the visible part of the upper incisor in the rat. They measured eruption rates subsequently for up to 24 weeks, then sacrificed the animals and examined them radiographically and histologically. Their findings were:-

1. The rate of eruption of the cut tooth doubled, approximately.
2. Radiographs showed an increase in all dimensions of the cut tooth and a decrease in the thickness of the dentine.
3. Microscopic studies showed (a) thinning of the dentine wall,
   (b) persistence of tall ameloblasts to gingival level,
   (c) a reduction in the width of the periodontal membrane.

They/

They concluded from this that, although the rate of eruption had increased, the functional rate of odontoblasts and ameloblasts was unchanged.

TAYLOR & BUTCHER (1951) showed that reducing the cross-sectional areas of the incisor of the rat increased its rate of eruption, the greater the amount of tissue removed the greater the increase in eruption rate. They also repeated the experiments of SCHOUR & MEDAK (1951) and showed that cutting one incisor out of occlusion increased the rate of eruption of that tooth.

NESS (1956) described a series of experiments in which the effects of shortening one or both rabbit mandibular incisors were investigated very thoroughly. He used the method which he had devised of measuring eruption rate radiographically in rabbits. His findings were:-

1. The increase in eruption rate of the unopposed rabbit mandibular incisor is roughly $2\frac{1}{2}$ times the mean "resting" rate.

2. When these high values were graphed, the curve was not flat topped but showed a peak value attained seven days after the shortening. The other high values, both before and after them, were on the same level, with normal biological variation.

3. The uncut incisors had a decreased rate of eruption subsequent to shortening the adjoining tooth, which was most marked initially and the visible lengths of these uncut teeth were reduced in every animal.

4./

4. The interincisal distance increased during the period when one tooth was cut out of occlusion.

5. The increase in eruption rate subsequent to shortening occurs within 24 hours, which was the shortest measurement interval he used.

6. The increased eruption rate subsequent to shortening is not associated with any change in the position in the bone of the basal end of the pulp of the shortened tooth.

In another experiment NESS investigated the hypothesis that shortening of one incisor and its subsequent increase in rate of eruption was the result of an increase in the level in the circulation of a hormone promoting eruption (SHIBATA, 1929). He suggested that, if this were so, shortening the other incisor, during the period of high eruption rate of its neighbour, would result in a rise of its eruption rate to peak value immediately. Accordingly, he carried out this procedure on twelve rabbits. However, the increase in eruption rate of the second cut incisor followed the same curve as its fellow, reaching a peak figure 7 days after shortening. NESS argued that this makes the initial hypothesis unlikely.

Application of Mechanical Stress.

TAYLOR & BUTCHER (1951) applied a protractive force and a retractive force on the mandibular incisors of the rat by drilling a hole in one near the gingival margin, and in the other near the incisal edge, and stretching a rubber band between the holes. They also applied a retractive force by stretching a spring or a rubber band from the first mandibular molar to the incisor of the same side.

They found that the protractive force increased eruption rate, but only to the same level as occurs in unimpeded eruption.

Retractive forces of over 2 gm. were found to retard eruption, while forces of more than 5 gm. stopped it completely. Histological examination of these teeth showed vascular stasis within the pulp and pulp necrosis as a consequence of this.

In long standing cases of retraction, ankylosis of the tooth to the alveolar bone took place.

In some cases in which the retraction had been applied for up to 16 days, then removed, it was found that eruption recommenced, and reached normal levels within about 10 days. It was found that, in some of these cases, complete necrosis of the pulp had occurred. Where complete necrosis occurred, no regeneration of normal pulp tissue, e.g. odontoblasts, occurred if the enamel organ had undergone necrosis. This is in agreement with the concept that enamel epithelium acts as an inducer on connective tissue, causing it to differentiate to form odontoblasts and pulp cells.

NESS/

NESS (1956) prevented the eruption of the rabbit incisor by fixing a stainless steel pin through the socket wall and tooth. He observed the results of this operation histologically, finding crumpling and folding of the enamel and dentine at the basal end of the tooth. In some animals, the base of the socket had moved backwards.

Removal of the Pulp.

HERZBERG & SCHOUR (1941a) reported experiments in which the pulp was removed from the left mandibular incisor of the rat via an opening in the labial enamel. The pulp tissue was removed by barbed broaches. The effect of this procedure on the rate of eruption was assessed by marking the labial enamel of both the operated tooth and the unoperated right incisor at the same level. It was found from observations of these marks that the teeth erupted at identical rates.

In another group of animals, HERTWIG's sheath was removed from the left incisor via an extra-oral incision. The effects of this procedure were assessed in the same manner as above. Again the incisors, operated and unoperated, erupted at identical rates. Histological examination of these animals showed that, subsequent to removal of HERTWIG's sheath, no further appositional growth of dentine or enamel occurred.

HERZBERG & SCHOUR concluded from these experiments that the eruption of the teeth and the growth of enamel and dentine were distinct and separate processes.

BRYER/

BRYER (1957) repeated the experimental evaluation of the effects of pulpectomy, both partial and total, on the rate of eruption of the rat incisor.

His findings were that partial pulpectomy had no effect on the rate of eruption. In three animals, he attempted to remove as much pulp tissue as possible via an opening into the pulp chamber produced by cutting off the exposed portion of the tooth. In these animals there was a great reduction in rate of eruption, the total eruptive movement in one animal being 1.14 m.m. over a period of 22 days, compared with an average 20.48 m.m. in control animals for the same period.

He examined these teeth histologically and found that in the total pulpectomy group, there was gross distortion of the tissues in the incisal part of tooth, with a very thin layer of dentine present. At the basal end of the tooth, the tissues appeared fairly normal, the dentine included, but the dentine was folded, as was the enamel. He published a photomicrograph of a section of one of these teeth.

While it is not of sufficient magnification for certainty, it does appear that the dentine has been broken through by his instrumentation, at the deepest part of the penetration which is about half way along the tooth. Basal to this area, the tissues appear vital, while incisal to it they are necrotic.

It is possible that the sequence of events was as follows:-

1./

1. Penetration of the dentine by the instruments used to remove the pulp.

2. Production of a haematoma in the periodontium adjoining this penetration.

3. Resolution of this haematoma with the production of granulation tissue which would mature into fibrous connective tissue.

4. Calcification of this fibrous connective tissue with resultant ankylosis of the tooth.

This would explain the cessation of eruptive movements, if the original assumption were correct.

BRYER (1957) further investigated the effect of:-

1. removal of the basal portion of the pulp of the incisor, surgically, via an extra-oral approach,

2. exposure of the basal portion of the pulp and probing it to produce trauma and haemorrhage in the pulp,

3. fracture of the incisor tooth by cutting through the alveolus and tooth with a carborundum disc.

He gives no figures for the rates of eruption in these animals but states that:-

1. In both groups where the vascular supply to the teeth was disturbed surgically, there was an immediate and often profound decrease in the unimpeded eruption rate. This decrease was most marked in the group which had had their pulps probed.

2./

2. Amputation of the basal portion of the pulp produced initially a severe reduction in unimpeded rate of eruption, but this rate gradually increased so that, one week post-operatively, the operated incisor was erupting more quickly than the functional, unoperated right incisor.

3. The incisal fragment of the fractured incisor showed little movement immediately following the surgical procedure, but the rate of eruption then gradually increased until the fragment was exfoliated.

BRYER examined these teeth histologically and found:-

1. Where the basal portion of the pulp had been removed surgically, the incisal fragment contained necrotic pulp tissue, basal to which was a vascular connective tissue, the line of demarcation being an inflammatory border.

2. In the case of the surgically fractured incisors, the incisal fragment again contained necrotic material. The basal portion showed a continuation of growth in length as evidenced by compression and folding of the basal ends of the dentine and enamel. No eruptive movement of this fragment had occurred, as shown by its position relative to the fracture site.

BRYER/

BRYER concluded from these experiments, and from the others which he had carried out, which are described elsewhere, that tooth eruption was a vascular phenomenon, in that the rate of eruption was related to the vascularity of the tissues at its basal end.

KOSTLAN et alia (1960) investigated the effect of resection of the basal zone of the pulp of the rat incisor on the rate of eruption.

They resected the basal zone of the pulp of the lower incisor both bilaterally and unilaterally. They also investigated the effects of this resection histologically.

Their findings were:-

1. The incisors continued to erupt subsequent to resection of the basal zone of the pulp.

2. The rate of eruption of the operated incisor was usually, but not invariably, less than that of the unoperated control incisor.

3. Resection of the basal zone of the pulp resulted in a cessation of dentine, enamel and cementum formation, although in a few cases, revascularisation of the pulp had occurred and coarse secondary dentine deposited.

4. When the apical end of a resected tooth approached the oral opening of the alveolus, eruption ceased.

5. Subsequent to resection of the basal pulp in one incisor, the visible part of the tooth remained the same length as its unoperated control on the other side.

6./

6. The structure of the periodontal tissue attached to the tooth did not change, until the apical end of the incisor passed from the alveolus.

KOSTLAN et alia concluded from this that (a) the structure of the periodontal tissues is in a state of continuous transformation during eruption (b) in this transformation, a tension is developed in the periodontal tissues and that this tension is the cause of the eruption of the tooth (c) the speed of eruption is regulated reflexly via baroreceptors which register the height of occlusion of the teeth.

Their experiments provided no basis for such conclusions, and they must be regarded as purely speculative.

They did not discuss how the periodontal tissues can produce the tension which they think is the cause of eruption.

NESS (1957) reported on an experiment in which the left mandibular incisor of the rabbit was divided transversely at its middle and the basal half of the tooth pinned to its alveolus. He found that the incisor divorced from its growing base continued to erupt but at a much reduced rate (25 microns per day as against 214 microns per day in controls).

The Effects of Radiation.

MEDAK et alia (1950) investigated the effects on the rate of eruption of the rat incisor of doses of X-radiation from 500^{r} to 4000^{r} (Roentgen (r)). Each animal received a single dose of radiation to/

to the anterior part of the head. They found that doses up to 2000^{r} increased the rate of eruption slightly, although from the published data it is not possible to assess the significance of this finding. Doses of radiation above this markedly decreased the rate of eruption, and caused complete cessation of eruption in three weeks.

In a later paper (MEDAK et alia, 1952) they described the microscopic changes which occurred subsequent to these doses of irradiation. These were as follows:-

Low doses (500 - 2000^{r}) produced a transient oedema in the odontogenic zone of the pulp with damage to the odontoblasts in this area. This led to mild hypoplasia of the dentine later.

High doses (over 2500^{r}) produced severe oedema in the pulp and periodontal tissues which led to destruction of the odontoblasts and cessation of differentiation of new odontoblasts. The enamel organ changed into a tissue resembling reduced enamel epithelium. The pulp cavity became filled with masses of osteo-dentine. The periodontal membrane (unaffected by low doses) changed through a disappearance of the intermediate plexus. In a few cases, some odontogenic epithelium regenerated, and in time, a new incisor formed.

MEDAK et alia concluded from this study that the mechanism of eruption of teeth was provided by the pressure from cellular proliferation at the basal end of the tooth. They added that this proliferative pressure produced its eruptive movement by acting against the cushioned hammock ligament described by SICHER.

In/

In 1961, GOWGIEL published some serendipitous findings on eruption which occurred in an experiment designed to study the pathogenesis of osteo-radio-necrosis. For this purpose eighteen macacus rhesus monkeys were submitted to fractionated external radiation to the mandible and the maxilla. The dose levels were 4,500 to 7,500^{r} and the animals lived up to 3¼ years post-irradiation. At irradiation the animals were aged from 14 to 18 months, and had a complete deciduous dentition, with the permanent teeth at various stages of development.

It was found that, at all dose levels used, the irradiation completely inhibited all further crown and root development in the permanent teeth. This was shown by comparison of radiographs. Calcification of the crown was not inhibited, as some crowns calcified further subsequent to irradiation. The pulp chambers of all the teeth were smaller than usual and the apex closed in an almost linear manner at the level of tooth development at the time of irradiation.

The striking feature, however, was that the deciduous teeth were shed and the rootless permanent teeth erupted into full, functional occlusion. GOWGIEL thought that the rate and times of eruption were retarded, but no measurements of this were made.

When the animals were sacrificed, their jaws and teeth were examined histologically. It was found that there was an abrupt transformation from normal, pre-irradiation appearances of dentine to the subsequently produced tissue.

The/

The odontoblasts were destroyed and osteo-dentine produced subsequent to the irradiation. In a few teeth, eruption did not occur, and it was found that bony ankylosis had occurred between the osteodentine and the alveolar bone. The periodontal membrane had no abnormal appearances, although its extent was much reduced.

GOWGIEL concluded from this experiment, that proliferation of pulp tissue and growth of the root were not factors involved in the production of the eruption of teeth, and it would seem that his findings are fairly conclusive.

Summary of Review of the Literature.

It will be seen that in spite of the large amount of research which has been done into this problem, the mechanism of the process by means of which a tooth is made to move relative to its investing tissues has not been discovered.

From the review it can be seen that there is no known method of completely stopping eruptive movements, short of physically pinning the tooth to its socket. Similarly the only method of speeding the process of eruption is by administering thyroxin or a cortico-steroid. While some other experimental techniques have been shown by some workers to increase the speed of eruption, there is no general agreement on these.

From this review it will also be seen that it has been conclusively demonstrated that the secretions of the endocrine glands and some food factors, including vitamins, influence the rate of eruption but do not cause it.

From the evidence available, a number of theories have been postulated to account for the process of eruption and these were well described by AITCHISON (1950). These are:-

1. Elongation of the root; this theory was discussed under the heading of early theories on eruption.

2. Bone growth: this theory was discussed when BRASH's work was reviewed.

3./

3. Blood pressure: this was discussed when CONSTANT's (1900) paper was reviewed.

4. Epithelial path: this theory was put forward by WARWICK JAMES (1909) and is discussed with his work.

5. Cellular proliferation: reference has been made to this theory at various places in the review, chiefly under the heading of the cushioned hammock ligament.

6. Periodontal membrane tension theory: this is the most recent, having been put forward by KOSTLAN et alia, (1960) and discussed with their work.

With the evidence now available, the only theories which remain tenable are the blood pressure theory and the cellular proliferation theory.

It is, of course, entirely possible that the true explanation is quite different from any of these theories.

Original Investigations.

Introduction.

After consideration of the welter of evidence and supposition which has been reviewed in the first part of this report, two lines of investigation were selected and pursued.

The first investigation was a histological examination of a group of animals in which teeth were erupting in an attempt to clarify the description of the cushioned hammock ligament.

The second investigation was designed to provide additional information relevant to the blood pressure theory of tooth eruption. It was hoped originally to do this in two ways. The experiments were to be carried out on rats, and the effect, on the rate of eruption of the incisor, of increasing and decreasing blood pressure was to be measured. However it was found that the only reliable way of increasing blood pressure in rats was by carrying out a unilateral nephrectomy, implanting a tablet of desoxycortisone acetate intramuscularly and giving a 1% solution of sodium chloride as drinking water. This procedure was carried out on a number of rats but the metabolic disturbances produced were of such severity that any observed changes in eruption rate could equally well have been attributed to factors other than the rise in arterial blood pressure. Accordingly this part of the experiment was abandoned.

It/

It was found possible to decrease arterial blood pressure, and the effects of this on eruption rate were measured. Hypotension was to be achieved in rats by means of drugs and it was fortunate that a number of hypotensive drugs had recently become available. These drugs were primarily for use in cases of hypertension in man.

Of the fairly large numbers of hypotensive drugs available, it was decided that guanethidine and hydralazine were most suitable, as both had relatively long periods of action.

Histological Survey of the Cushioned Hammock Ligament.

Materials and Methods.

Thirty-four animals, the jaws of which contained actively erupting teeth, were prepared for routine histological examination. They included dogs, cats, sheep, pigs, a calf, guinea pigs, rabbits, rats and mice. Teeth were taken to be actively erupting if the crown had just penetrated the oral mucosa or if the mucosa was markedly bulged over the crown.

Nearly all the animals were obtained through the kindness of the Staff of the Royal (Dick) School of Veterinary Studies.

None were diseased in any way. They were fixed in 10% neutral formalin, processed in the normal way for histological examination and double embedded in celloidin and paraffin wax to minimise tissue distortion.

The sections were cut in the long axes of the teeth at a setting of six microns on the microtome. Serial section through the open apices were stained haematoxylin and eosin, van GIESON, MALLORY's anilene blue collagen stain (MALLORY, 1936) and GOMORI's silver impregnation method for collagen (GOMORI, 1939).

In the jaws sectioned, teeth were present at all stages of development and eruption and sections were cut through some hundreds.

Sections/

Sections of teeth were stained and examined at all stages of development, that is from the cap stage of the enamel organ, through enlargement of the enamel organ and then calcification of the crown, then the formation and calcification of the roots, and the eruption of the teeth. Both single and multirooted teeth of limited eruption were examined, and incisors and molars of continual eruption.

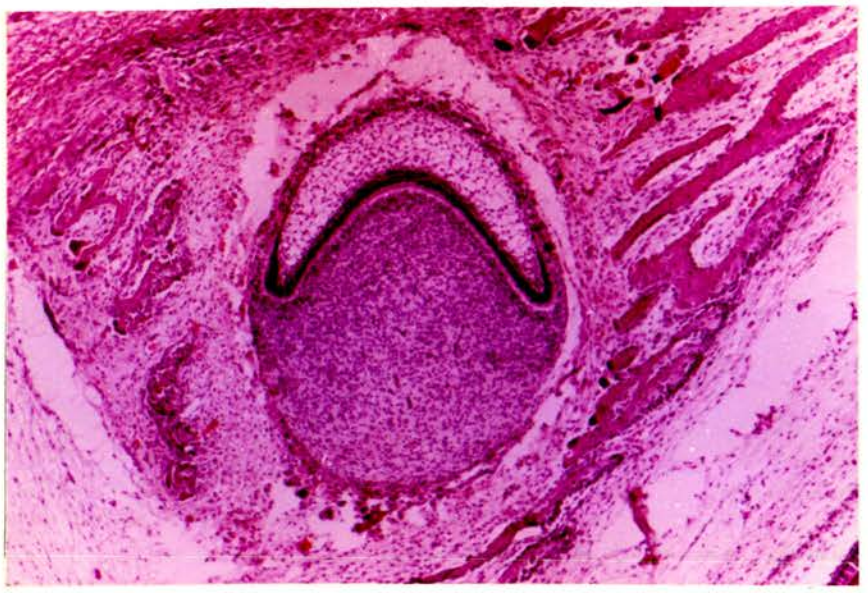


Fig. 6.
Tooth germ of mandibular permanent incisor; dog, age 3 weeks, H. & E., X 32.

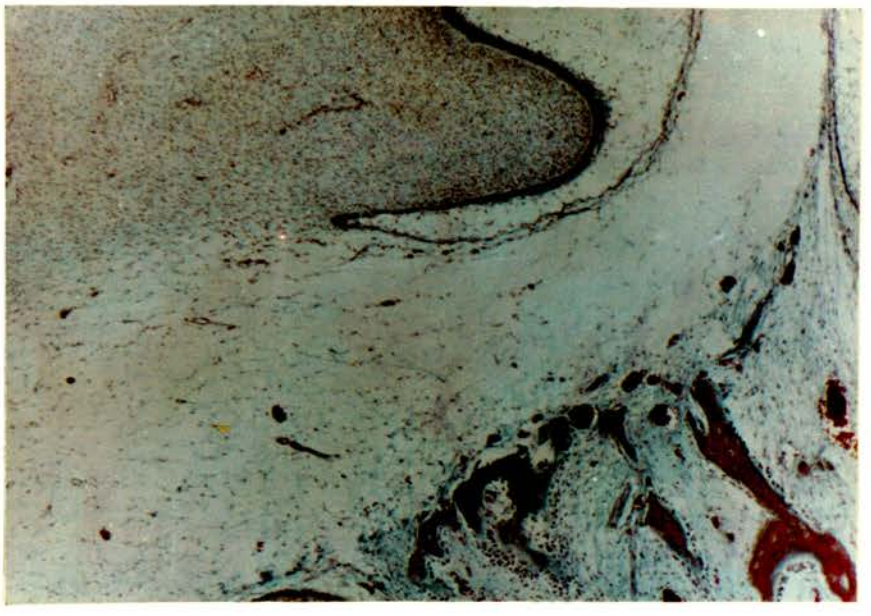


Fig. 7.
Base of follicle of mandibular deciduous canine; pig, age 15-17 weeks in utero; Mallory, X 32.

Observations.

1. Teeth of Limited Eruption.

Cap Stage of the Enamel Organ.

Starting observations, somewhat arbitrarily, at this stage of development, the follicular tissue was seen to be composed of simple, relatively undifferentiated mesenchymal cells, the appearance of which was the same throughout the width of the follicle, from the surface of the dentine papilla to the alveolar bone (Fig. 6). Further, this tissue was identical with the osteogenic mesenchyme which surrounded the tooth germ. It was a fairly cellular tissue, but no collagen fibres could be detected.

The bone itself at this stage was in a constant state of flux. In general, resorption could be seen occurring on the mesial walls of the alveoli, deposition on the distal walls, although this was not invariable.

Bell Stage of the Enamel Organ.

With the development and enlargement of the enamel organ ultimately reaching the "bell" shape, changes occurred in the follicular tissues. The bulk of the follicle became less cellular, numerous intercellular spaces developed and there was a total absence of any fibrillar structure/

Fig. 8.
Base of follicle of mandibular deciduous molar; dog, age 3 weeks; van Gieson, X 32.

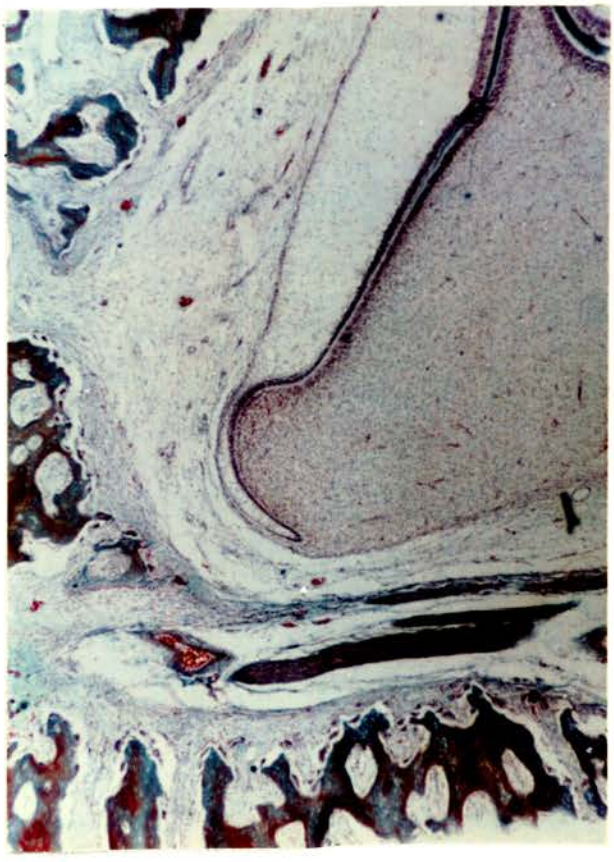


Fig. 9.
Mandibular deciduous molar; dog, age 4 weeks; Mallory, X 20.

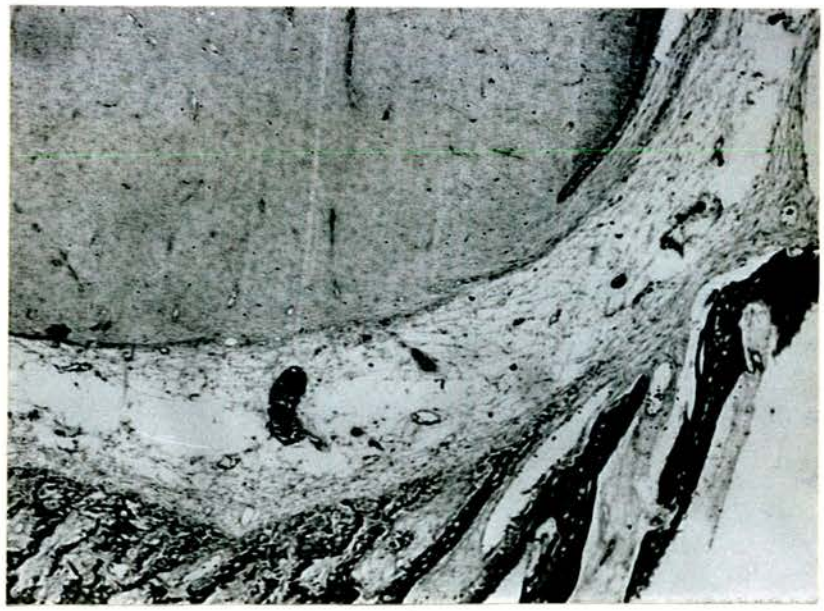


Fig. 10.
Maxillary deciduous incisor; dog, age 4 weeks;
Gomori, X 32.

structure, in other words, the follicular tissue became loose, ordinary, areolar tissue (Figs. 7 & 8).

However, the follicular tissue immediately adjoining the alveolar bone retained its original structure, so that in comparison with the areolar tissue forming the bulk of the follicle, it appeared more cellular and more fibrillar with the fibres lying parallel to the alveolar wall. This tissue was, of course, periosteum (Figs. 7 & 9).

At this stage also, in relation to the cheek teeth, it was found frequently that the base of the dentine papilla was separated from the dental vessels and nerve by the areolar tissue only, no bone being present in the base of the crypt (Fig. 8).

Stage of Crown Calcification.

The appearances of the areolar tissue and periosteum remained unchanged during this period (Fig. 9).

Towards the end of the period a few fibres were seen to form in the follicle close to the surface of the tooth germ with their long axes parallel to the circumference of the germ (Fig. 10). They could be traced up the sides and over the enamel organ although they were relatively sparse in this area and not particularly closely applied to the enamel organ. The fibres were closely applied to the base of the pulp, as it now was, but they did not completely cover it, as sections cut through the centre of the open apex showed them to be absent centrally.

This/

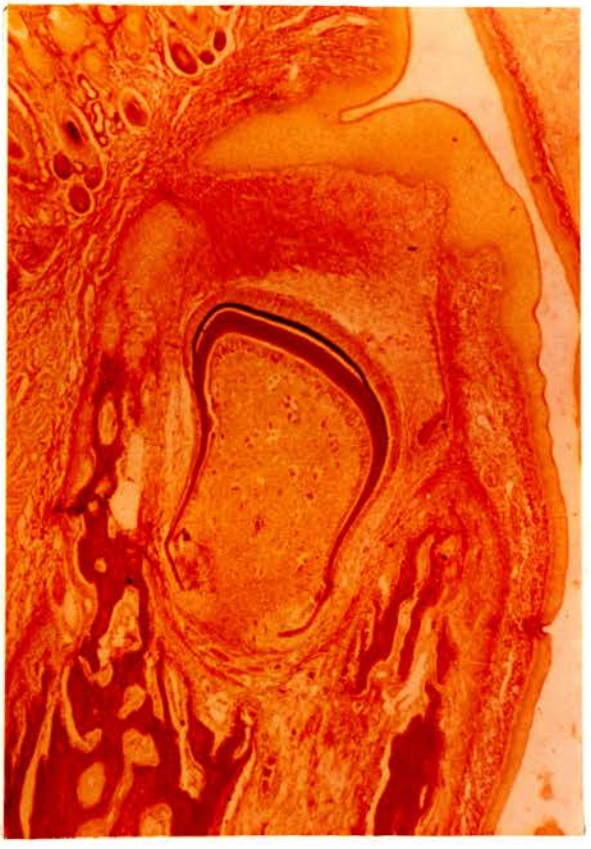


Fig. 11.
Mandibular deciduous incisor; cat, age 1 day; van Gieson, X 20.

Fig. 12.
Mandibular deciduous incisor; cat, age 1 day; van Gieson, X 32.

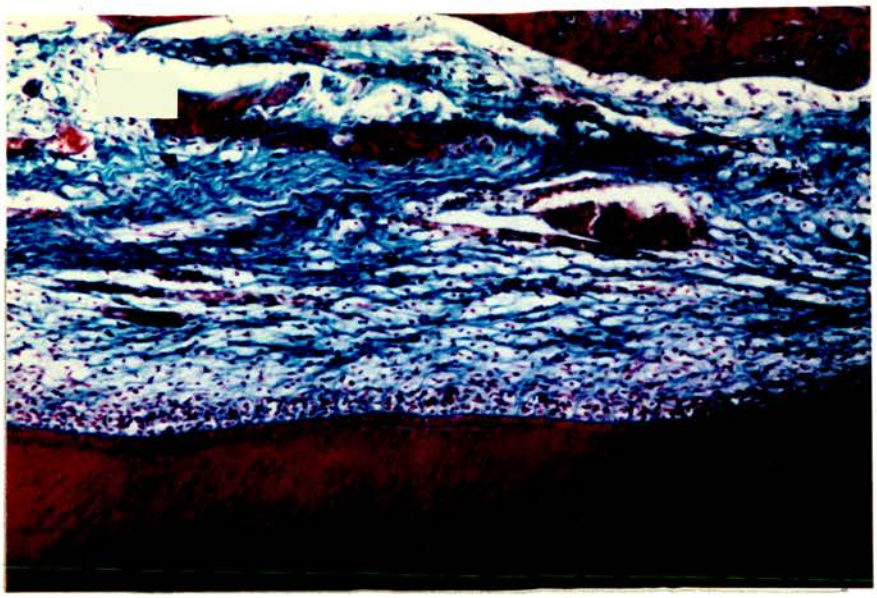


Fig. 13.
Mandibular deciduous molar; calf, age 1 day;
Mallory, X 80.

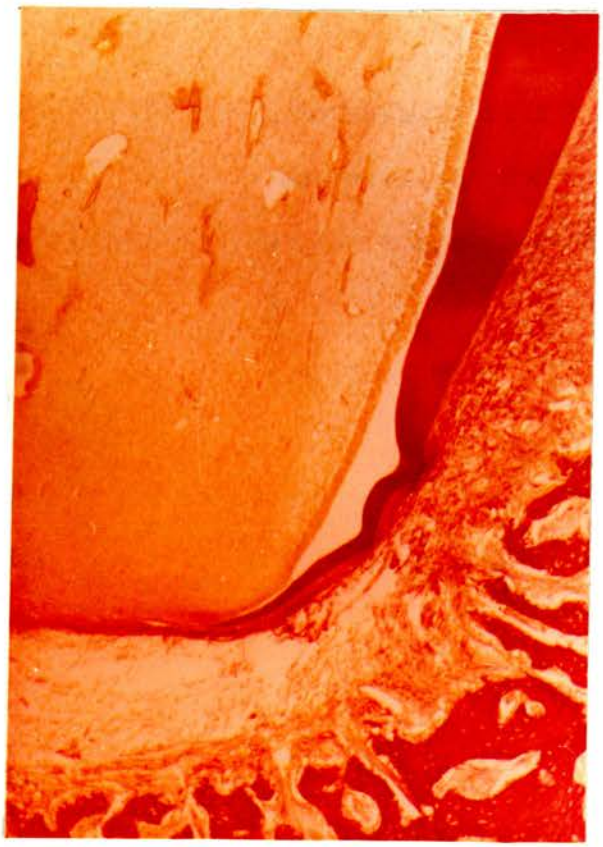


Fig. 14.
Mandibular permanent incisor; dog,
age 6 months; van Gieson, X 32.

This band of fibrous tissue had collagen fibres in it. It began to appear when calcification of the crown was well advanced and became more obvious during calcification of the root. It was not attached to the alveolar bone and resembled a capsule surrounding the tooth germ.

The part lying across the base of the pulp will be referred to as the pulp limiting membrane. This structure was never seen round multi-rooted teeth, its occurrence being limited to those with single roots.

In some incisor teeth at this stage, and only in incisor teeth, a more fibrous type of capsule developed in which the fibres could be seen throughout the entire thickness of the follicle. The fibres took the stains for collagen (Figs. 11 & 12).

The density of the fibres varied considerably. They were most obvious around mandibular, deciduous incisors and the density varied also between species, being greatest in the cat, of the species examined. However, this type of follicle was also seen in pigs, dogs and sheep, and around maxillary incisors also, with varying degrees of density of the collagen.

The attachment of the fibres was not to the alveolar bone but to the more massive condensation of mesenchyme which had been laid down superficial to the follicle to form the lamina propria of the oral mucosa (Figs. 11 & 12).

In/

In some cases the fibrous type of follicle was present only on the labial aspect of the tooth, while lingually the follicular tissue was areolar.

This appearance rapidly changed when the formation of cementum began.

Stage of Calcification of the Root.

In every case where there was macroscopic evidence of active eruption taking place, that is where the overlying mucosa was bulged or where one cusp or more had penetrated the mucosa, microscopic examination of the section showed that cementum had been formed and some periodontal membrane with its characteristic fibre arrangement was present. This was an invariable finding.

The converse to this was not found invariably. That is to say, microscopic examination showed that in some cases cementum and periodontal membrane had formed around teeth which had had no macroscopic signs of being in a state of active eruption. In these cases, however, the teeth were all situated deep in the jaws.

The changes in the mesenchymal tissue of the follicle which occur to form the periodontal membrane, must take place rapidly, as no sections were found in which cementum was present but no periodontal membrane or vice versa.

The/

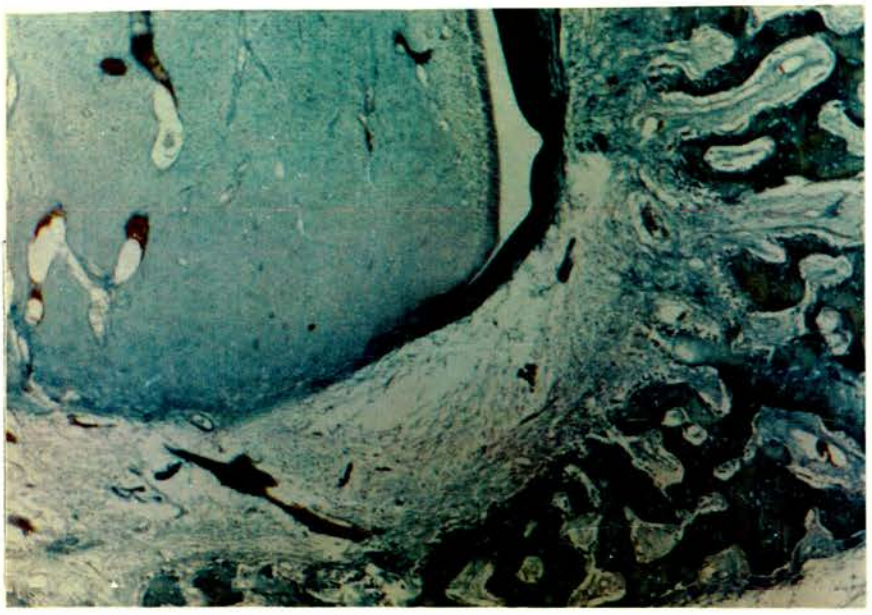


Fig. 15.

Mandibular permanent incisor; dog, age 6 months; Mallory, X 32.

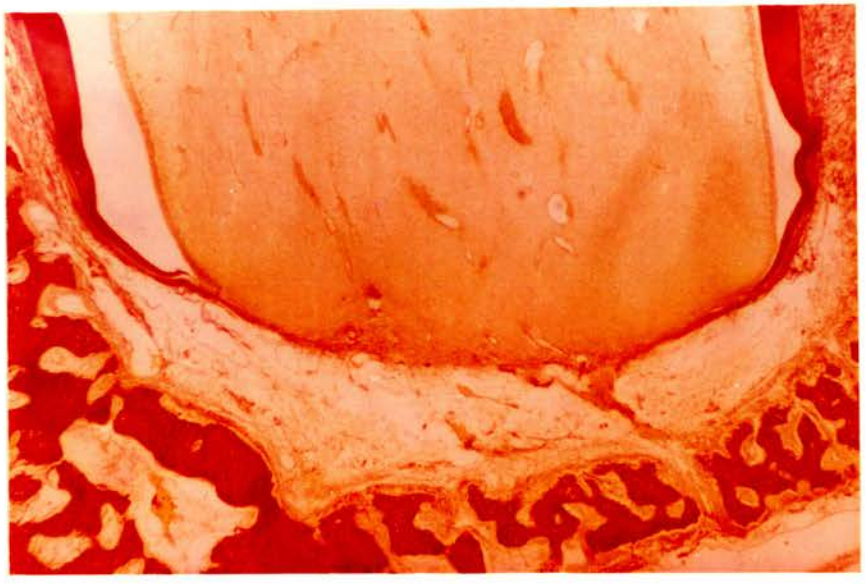


Fig. 16.

Mandibular permanent incisor; dog, age 6 months; van Gieson, X 20.

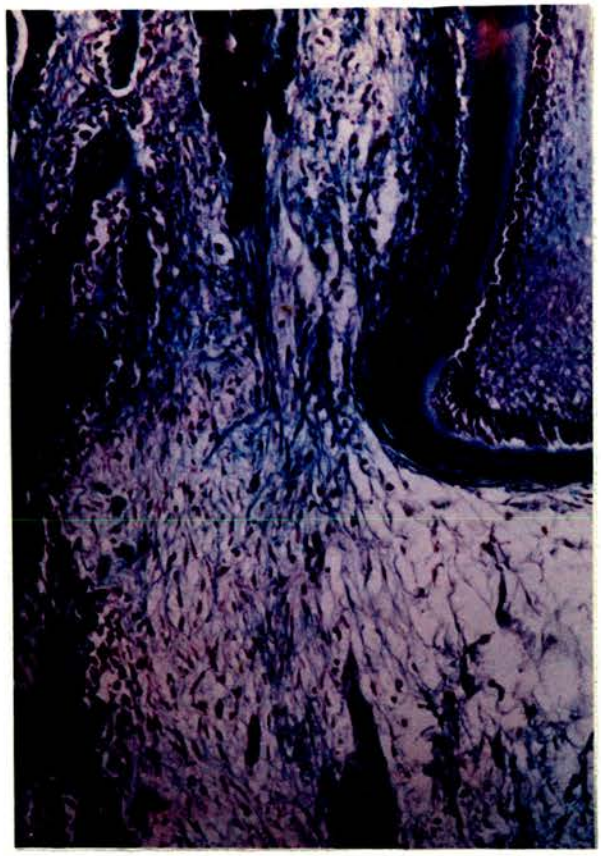


Fig. 17.
Mandibular deciduous incisor; pig, age 5 weeks; Mallory, X 80.

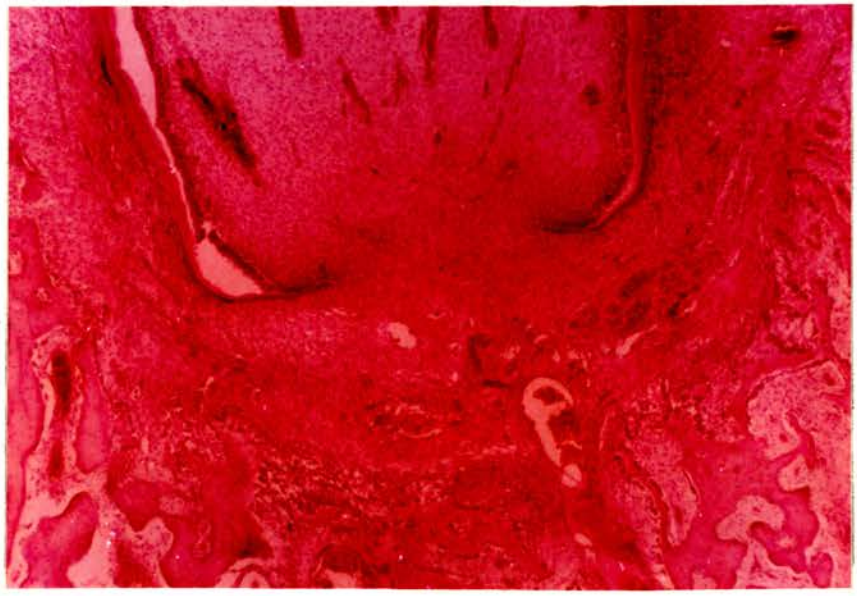


Fig. 18.
Maxillary deciduous canine; pig, age 3 weeks; H. & E., X 20. (Tooth in occlusion).

The periodontal membrane itself showed the three strata usually described, these were:-

1) a fairly narrow zone adjacent to the cementum in which the fibres could be seen to be embedded in the cementum.

2) a much wider zone adjacent to the alveolar bone in which the fibres could be seen to be embedded in the bone. Many blood vessels could be seen in the zone.

3) a central zone, closer to the tooth than the bone, in which the fibres were not directly attached to bone or cementum but intermingled with the fibres from zones 1) and 2). This was the "intermediate plexus".

These appearances remained the same throughout the period of eruption of the tooth (Fig. 13).

The periapical tissues of single rooted teeth at this stage were the same as during the period of calcification of the crown, that is, they consisted of a thin pulp limiting membrane, which was by now more obvious, a much wider zone of areolar tissue, and a layer of periosteum (Figs. 14 - 17). The pulp limiting membrane still did not completely cover the base of the pulp, being absent in the middle (Figs. 14 - 16).

The fibres of this membrane when followed up the side of the tooth could be clearly seen to intermingle with the fibres of the inner and middle zones of the periodontal membrane. In no case were they attached to the bone of the alveolus (Fig. 14).

When/

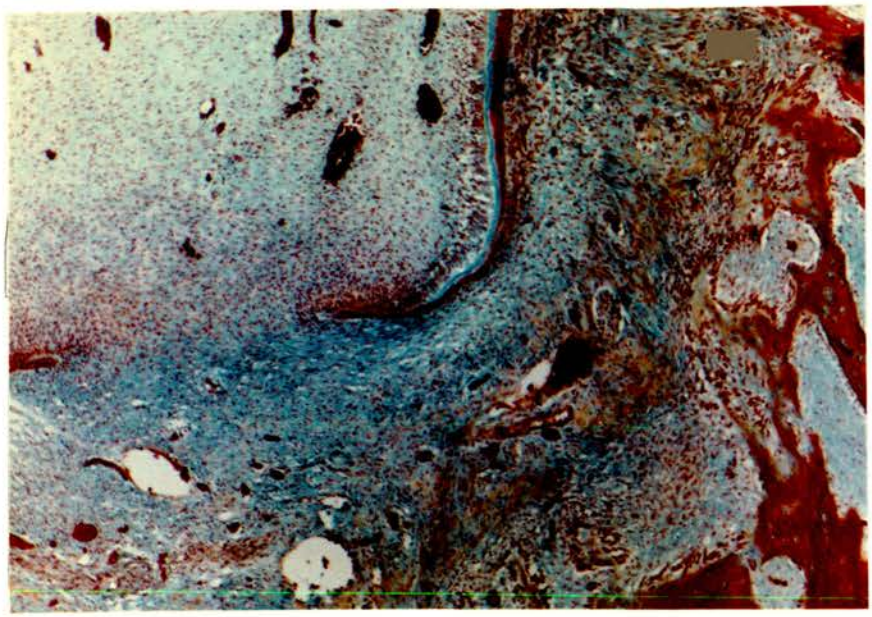


Fig. 19.

Maxillary deciduous canine; pig, age 3 weeks; Mallory, X 32.

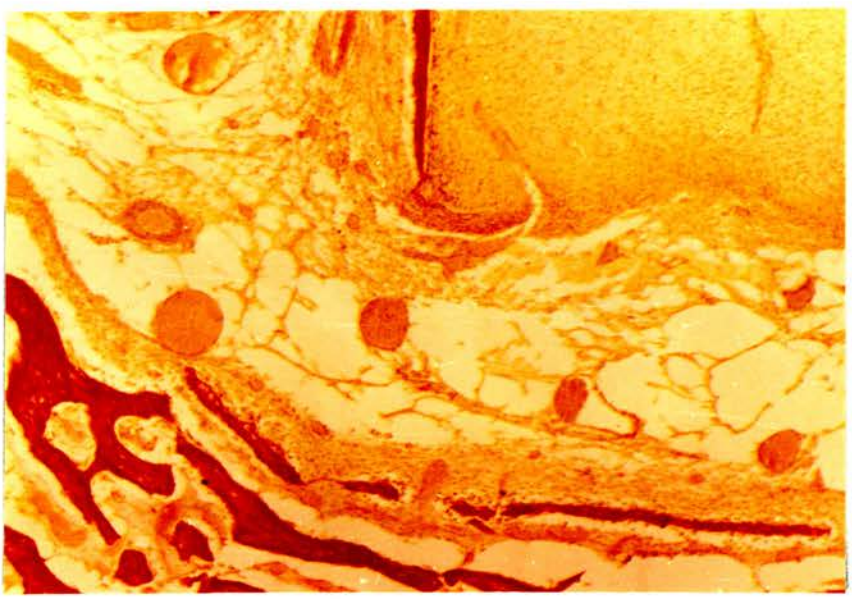


Fig. 20.

Mandibular deciduous molar; calf, age 1 day; van Gieson, X 32.

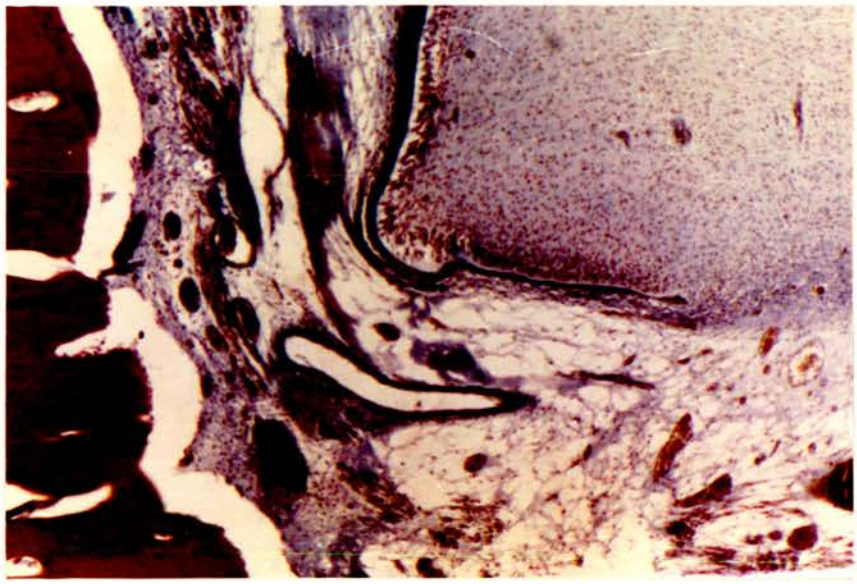


Fig. 21.

Mandibular deciduous molar; calf, age 1 day; Mallory, X 32.

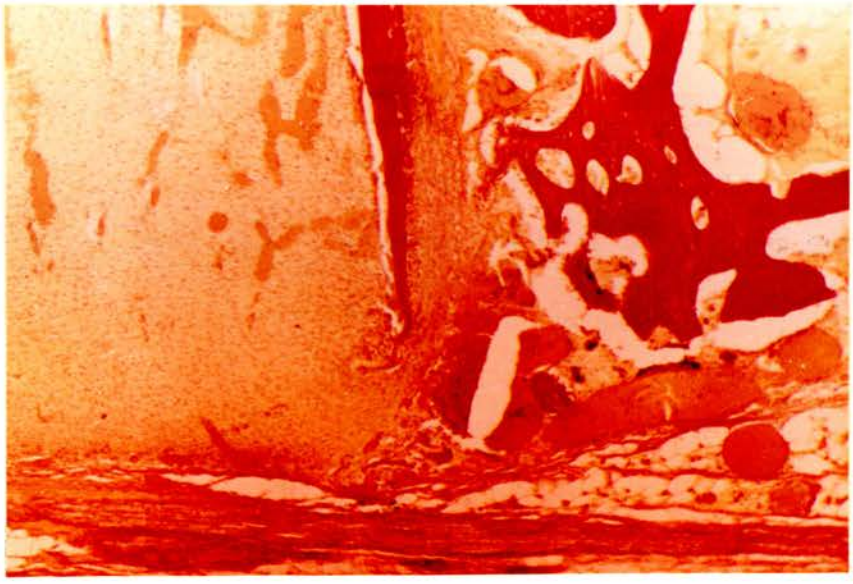


Fig. 22.

Mandibular deciduous molar; sheep; age 3 weeks; van Gieson, X 20.

When the period of active eruption was nearly complete, a general condensation of the areolar tissue occurred, the entire periapical tissue becoming much more dense and collagenous (Figs. 18 & 19). It seemed that this change took place immediately after the tooth came into functional occlusion, as it was only seen in such teeth.

In the case of multi-rooted teeth, the pulp limiting membrane was completely absent, and the periapical tissues consisted of the areolar tissue which was often very vascular, and the periosteum (Figs. 20 - 22).

Quite frequently the base of the pulp in molars and premolars lay in close approximation to the inferior dental canal with no bone separating the pulp from the nerve and blood vessels (Fig. 22).

In other instances, in incisors, canines and cheek teeth, new bone trabeculae were being laid down in the fundus of the alveolus. In no instance, was there any evidence of bone resorption in this area.

Fig. 23.
Mandibular incisor; rabbit, age 2 days; H. & E., X 32.

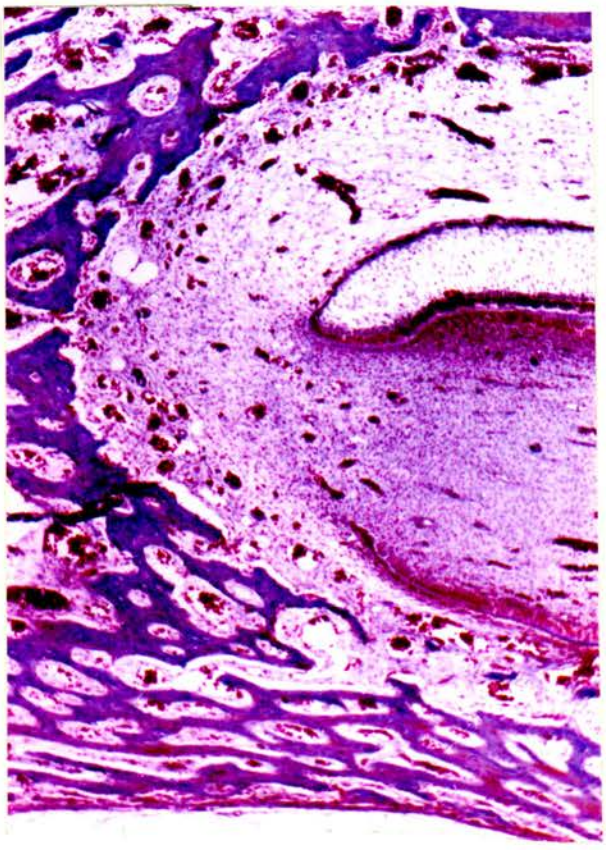


Fig. 24.
Mandibular incisor; rabbit, age 2 days; Mallory, X 32.

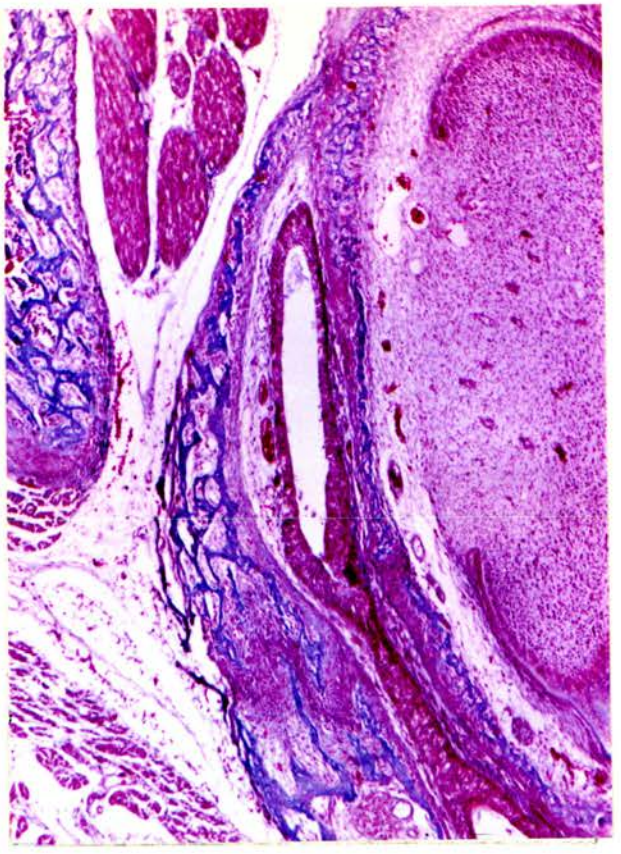


Fig. 25.
Maxillary incisor;
rat, age 8 days;
Mallory, X 32.

Fig. 26.
Mandibular incisor;
rat, age 3½ months;
van Gieson, X 80.

2. Teeth of Continual Eruption.

Incisors.

The periapical tissues from this class of teeth were examined in rats, mice, rabbits and guinea pigs, and in both young and adult animals.

In all cases these tissues were found to consist of two zones only - a layer of periosteum and a much broader zone of areolar tissue (Figs. 23 - 25). However in adult animals the appearances of the cells in part of the areolar zone were quite different from those found in any of the other classes of teeth (Fig. 26). The difference lay in the arrangement and density of the cells in the layer immediately adjoining the base of the pulp. These cells were elongated, orientated with their long axes at right angles to the long axis of the tooth and were closely packed, with little intercellular material. They formed a layer some six cells thick and were quite distinct from the bulk of the periapical areolar tissue, although the two zones merged together.

When stained by silver impregnation (Fig. 27) this area could be seen to have a definite reticulin network in which the fibres were parallel to the long axes of the cells. These fibres had no attachments at the periphery of the base of the pulp - they simply stopped. When stained for collagen (Fig. 26), only slight traces could be detected here and there between the cells. There was no continuous or annular sheet as found in relation to single rooted teeth of limited eruption.

This/

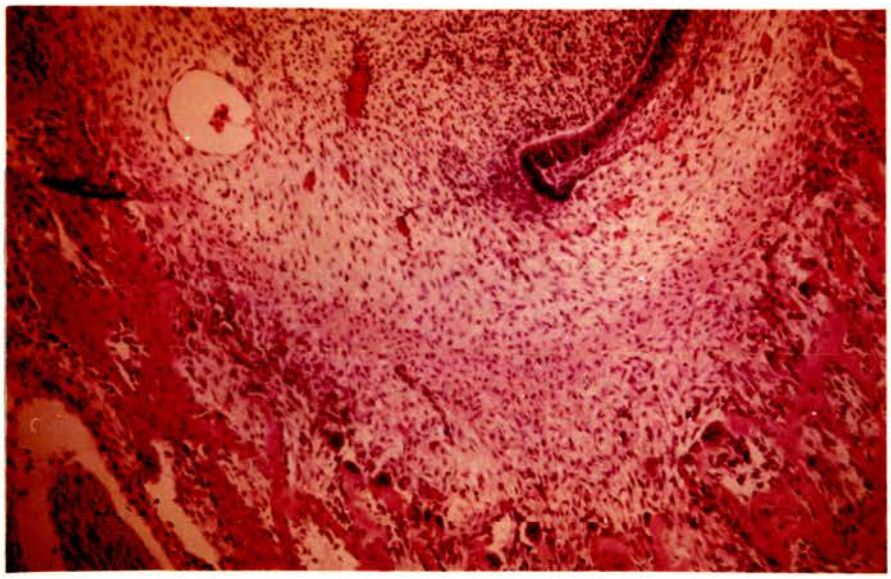


Fig. 28.

Base of alveolus of mandibular incisor; rat, age 10 days; H. & E. X 32.

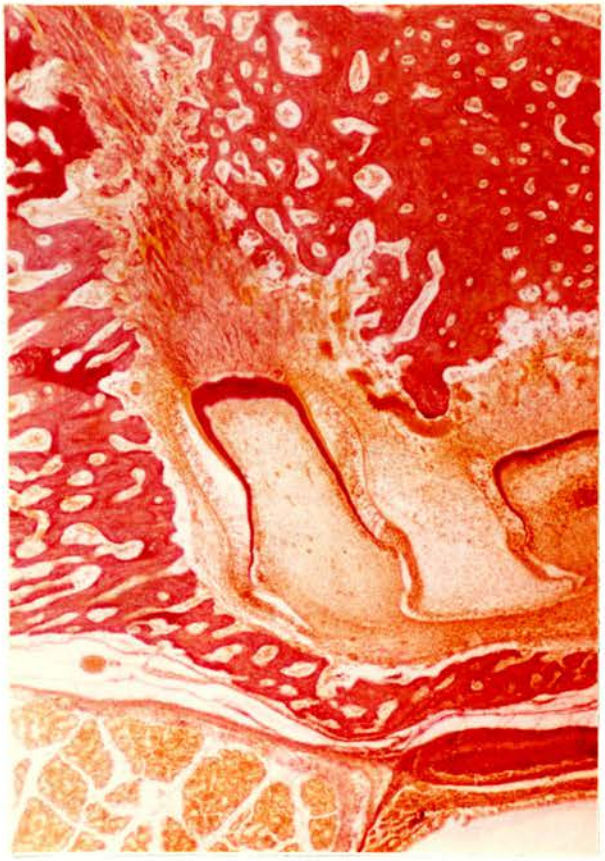


Fig. 29.

Maxillary molar; guinea-pig, age 1 day; van Gieson, X 20.

Fig. 27.
Mandibular incisor; rat, age 3½ months;
Gomori, X 20.

This layer of closely packed, uniformly arranged cells, was also present in young animals but the uniformity of arrangement was less marked than in the adults and the line of demarcation between these cells and the broader zone of areolar tissue was less distinct.

The layer was most obvious in the rat, the cells being less closely packed with more diversity of orientation in mice, rabbits and guinea pigs. In the latter group also, the reticulin fibres were more divergently arranged than in the rat.

A unique feature seen in the young specimens of these animals was resorption of bone in the base of the alveolus. This was again most obvious in the rat (Fig. 28) where very many osteoclasts were seen. Although osteoclasts were present in all sections examined in the other animals of the group, they were much less frequent.

Molars.

These teeth were examined in young and old rabbits and guinea-pigs. The appearances were similar to those found under multi-rooted teeth of limited eruption, that is only periosteum and loose areolar tissue were present. No cellular condensation of the type seen in relation to continuously erupting incisors was found (Figs. 29 - 32).

Discussion/

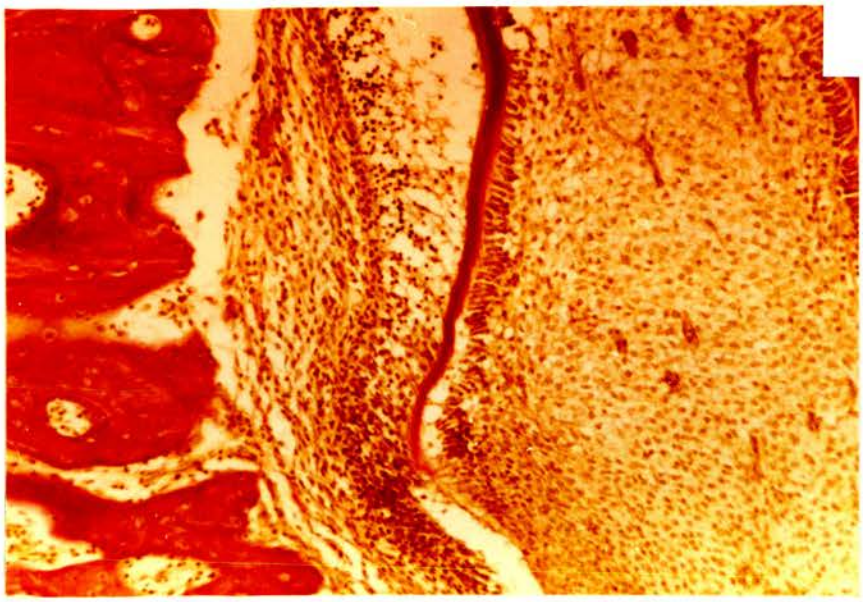


Fig. 30.
Maxillary molar; guinea-pig, age 1 day;
van Gieson, X 80.

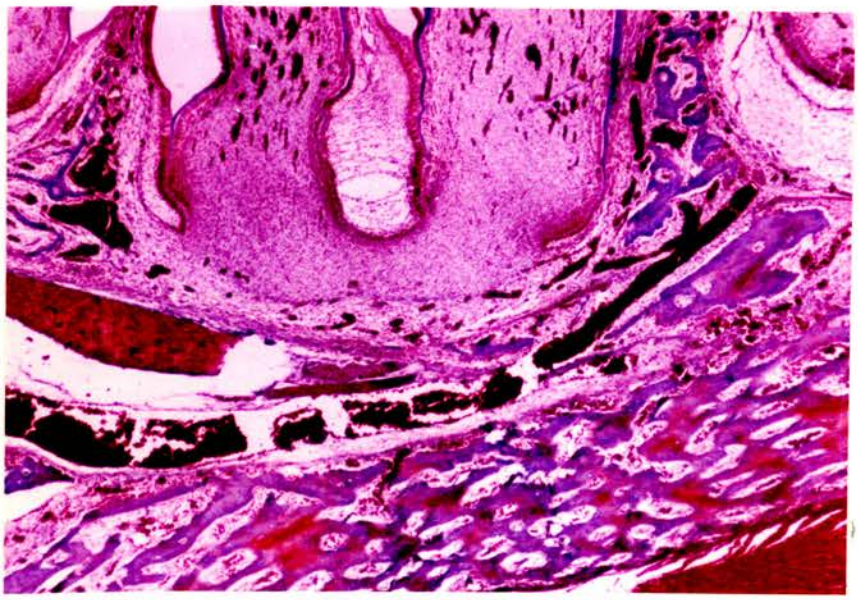


Fig. 31.
Mandibular molar; rabbit, age 2 days;
Mallory, X 20.

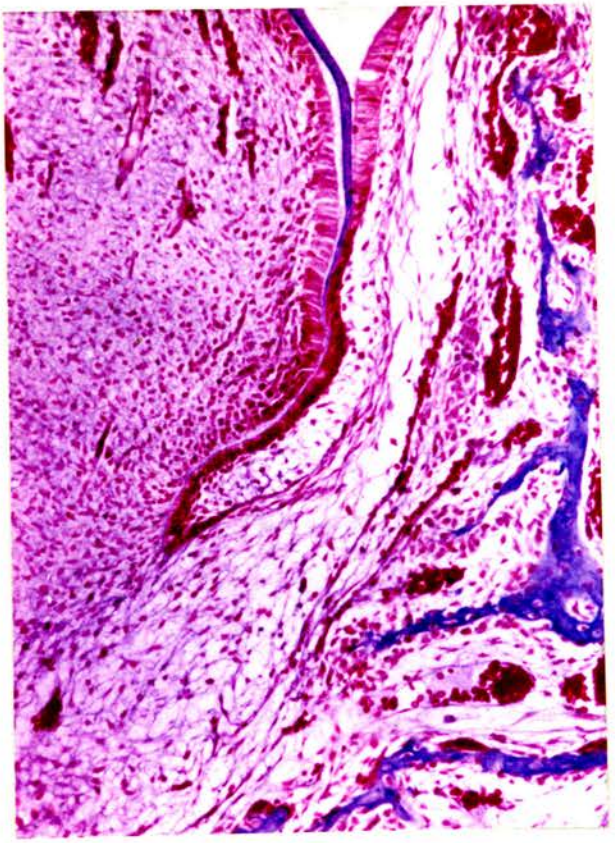


Fig. 32.
Mandibular molar; rabbit, age 2 days;
Mallory, X 80.

Discussion.

The original object of the work was to describe more fully the cushioned hammock ligament but it was found in practice that this structure could not be seen at all.

As was related in the review of the literature, the hammock ligament has been described in the past by two investigators, SICHER (1942a & b), and SCOTT (1953). Sicher called the structure the "cushioned hammock ligament", Scott the "hammock ligament", and their description of it did not tally exactly.

To consider the work of Sicher first, it is necessary to bear in mind the functional significance which Sicher attributed to this ligament. He described it as occurring in relation to only single-rooted teeth of limited eruption, and in relation to continuously erupting teeth of all types, single and multi-rooted.

In regard to single-rooted teeth of limited eruption, it is clear that the structure which Sicher called the cushioned hammock ligament is the annular sheet of fibrous tissue which has been called the pulp limiting membrane in this account.

Now a hammock, by definition, is a net hung by its ends. To call this membrane a hammock slung across the base of the tooth and to state that the tooth can move away from it (by whatever means are postulated to account for the movement) as Sicher did, makes it necessary for this membrane to be attached to the vertical wall of the alveolus directly, and/

and separate from the attachment of the periodontal membrane, the implication of the presence of a line of cleavage being obvious. Indeed Sicher illustrated just this point in a diagram in his report (1942b). Given the functional implication, then certainly the membrane must not be attached to the innermost layer of the periodontal membrane which moves with the tooth. Otherwise when the tooth moved relative to the bone, as it is known it does (NESS & SMALE, 1959) then the membrane would move with it.

Now it was invariably found in this study that this fibrous membrane was attached to the periodontal membrane, and to its innermost layer at that. It was never found to be directly attached to bone, and no line of cleavage between membrane and periodontal membrane was found.

To describe this membrane, therefore, as a hammock ligament seems inappropriate.

Sicher further described this ligament as "cushioned", stating that it contained fluid droplets which he thought were "mucoid". He stated that this tissue, the fibrous material and the fluid, was unique in the human body. His explanation for its existence was that the fluid droplets rendered the tissue incompressible, so that the pressure produced by growing bone in the base of the alveolus could provide an additional force to produce axial movement of the tooth.

Disregarding the somewhat dubious argument on imcompressibility, it was found in all the specimens examined that the tissues were perfectly ordinary periosteum, loose areolar tissue, and thin, but dense, collagenous/

collagenous fibrous tissue. No special histochemical techniques were employed to investigate the fluid droplets, as none were found. This finding is in agreement with ADAMS (1960) who found no unusual fluid droplets in this area in the cat.

The findings in this survey on multi-rooted teeth of limited growth are in agreement with Sicher's work. No fibrous membrane was found across the base of the pulp of these teeth.

Turning now to incisors of continual eruption, in this study no hammock ligament could be seen. In the adult rat incisor when stained by silver impregnation, a fibrillar layer could be seen in the periapical tissues lying across the base of the pulp. When this area was stained for collagen, only a very few, thin fibres could be detected, and again these were not attached to the alveolar bone in such a way as to resemble a hammock. So indistinct were the collagen fibres that it seems exaggerated even to call them a membrane, far less a ligament.

In continually erupting molars, no collagen fibres at all could be detected lying across the base of the pulp.

These findings are in agreement with those of NESS & SMALE (1959) who worked on the rabbit incisor and those of HUNT (1959) who investigated the molar of the guinea-pig.

SCOTT's (1953) description of the hammock ligament occurring under multi-rooted teeth of limited growth was not supported and, as has been said before, no hammock ligament was found under single-rooted teeth either.

The/

The findings of ECCLES (1959 & 1961) are in general agreement with the findings in this study.

The significance of this study is that, as the hammock ligament has been shown to lack the characteristics of a hammock, then it cannot act in the way Sicher and Scott suggested - that is to transmit the pressure derived from the proliferation of pulp cells to the alveolar bone as tension, thereby preventing bone resorption and resulting in axial movement of the tooth.

Furthermore the fact that this membrane was found in single but not multi-rooted teeth and not in teeth of continual eruption makes it unlikely that it plays any role in eruption, on the a priori grounds of reasoning that one mechanism of eruption for all teeth is more likely than a different mechanism for different types of teeth.

Conclusion.

The cushioned hammock ligament did not exist in any of the specimens examined. The tissues found around the apex of developing and erupting teeth have been described.

The Effects of Reduction in Arterial Blood Pressure on the Rate of Eruption of the Rat Mandibular Incisor.

Introduction.

The object of this experiment was to observe the effect on eruption rate of a marked reduction in arterial blood pressure. It was hoped to maintain this reduction in pressure for a period of some days by means of hypotensive drugs, and it was hoped to alter the capillary pressure as well as the arterial pressure as the recent proponents of the blood pressure theory of eruption had suggested that this acted via the small vessels of the pulp and periodontal membrane (BRYER, 1957; NESS, 1959).

As information of the action of hypotensive drugs on normotensive rats was not available, it was necessary first to demonstrate that reduction in arterial blood pressure and an alteration in capillary pressure were produced by the drugs to be used.

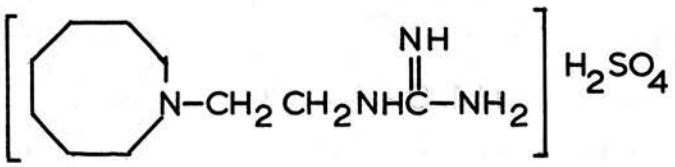
Experiment 1.

Object: To measure the effects of hydralazine and guanethidine on the arterial blood pressure of normotensive rats.

Materials and Methods:

1. **Drugs.**

* Guanethidine was synthesised by MULL and his co-workers in 1957. Chemically it is (2 - (octohydro - 1 - azocinyl) ethyl) guanidine sulphate.



It is a white, crystalline powder, soluble in water. Its pharmacological properties were studied by MAXWELL et alia (1960). They concluded that its prolonged hypotensive action was due to chronic interference with the release and/or distribution of the neurohumoral transmitter at the sympathetic neuromuscular junction.

Guanethidine markedly lowered blood pressure in neurogenic and renal hypertensive dogs. The lowering of pressure was less marked in normotensive dogs. It antagonised the hypertensive action of amphetamine and the pressor response to bilateral occlusion of the carotid arteries.

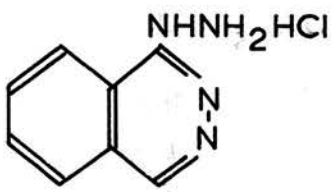
The/

The preliminary response to the drug simulated sympathetic stimulation, but this lasted less than 3 hours. From 10 to 30 minutes after the intravenous injection of 15 mgm/kg. of guanethidine in dogs there occurred a rise in blood pressure, panting, piloerection, occasional emesis and diarrhoea. Following these changes, a fall in blood pressure is found and during this period there is a diminished response of the nicitating membrane to both pre- and post-ganglionic stimulation. Electrical recording from the post-ganglionic nerve during pre-ganglionic stimulation shows that, although conduction through the ganglion is sometimes depressed, much larger doses are required than for inhibition of the nicitating membrane. The main site of action is thus thought to be distal to the ganglion (ROBSON & STACEY, 1962).

No gross or microscopic abnormalities in the principle organs of dogs followed 20 - 40 mgm/kg. guanethidine orally every day for 30 days.

It has been widely used clinically in the management of hypertension.

Hydralazine* was introduced by GROSS, DRUEY & MEIER (1950) as a hypotensive drug. Its chemical structure is:-



1. - hydrazinophthalazine.

It is used as its hydrochloride which is a pale yellow crystalline powder, soluble in water.

It/

It was widely used in the treatment of human hypertension but undesirable side effects were noticed and its clinical use has been discontinued. The toxic effects were investigated by GARDNER (1957) who described them as essentially multifocal hepatic necrosis, but these developed only after administration of the drug for some weeks.

Its mode of action remains obscure, but appears to have several components. It acts centrally on the mid-brain (CRAVER et alia, 1951) and diminishes the outflow of sympathetic, vasomotor discharges. It also acts on the peripheral vascular elements and inhibits the action of adrenaline and nor-adrenaline in perfusion experiments and in vitro (KIRPEKAR and LEWIS, 1957). It also antagonises 5 - hydroxytryptamine to some extent. It does not antagonise acetylcholine, but potentiates it in most circumstances.

Its action in vivo is essentially to abolish vasoconstriction in vascular beds, irrespective of the nature of the agent producing the constriction.

It reduces systolic arterial blood pressure in rats with renal and steroid hypertension (GARDNER, 1960).

No information was available in the literature of the action of either of these drugs on arterial pressure in normotensive rats.

These two drugs were chosen because both had fairly long periods of action and they seemed to produce hypotension by different mechanisms.

Material/

* The guanethidine and hydralazine used in this and subsequent experiments were obtained through the generosity of A.B. Tattersall, Esq., of Ciba Laboratories Ltd., Horsham, Sussex.

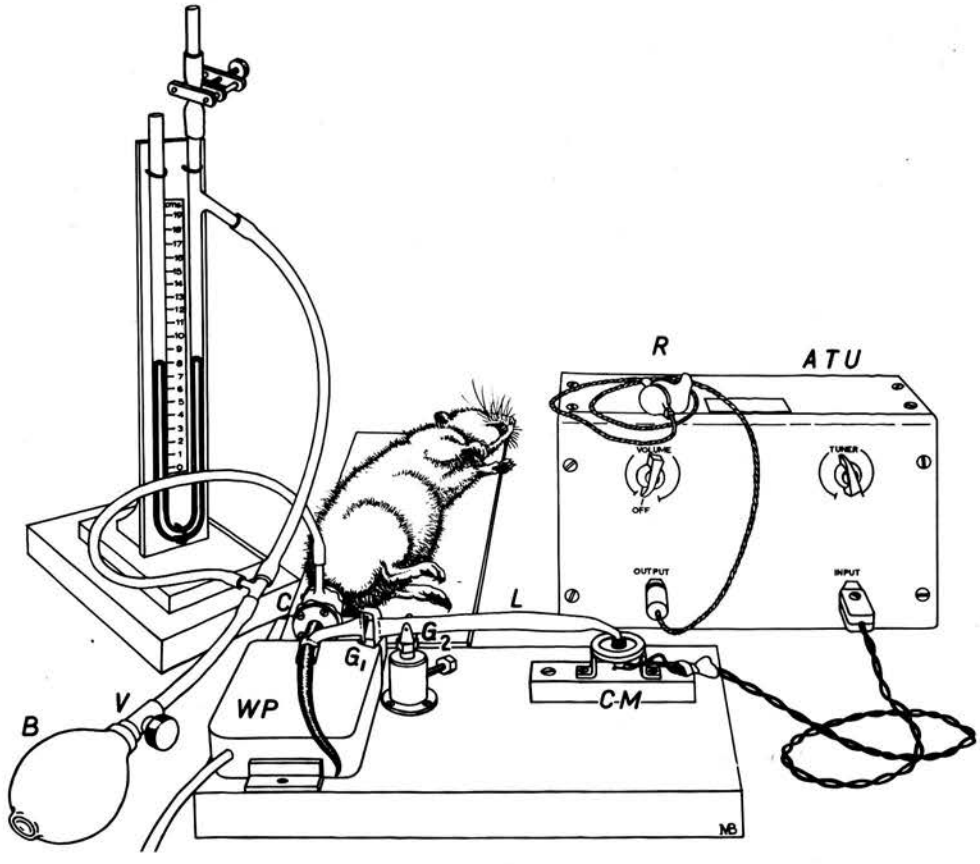
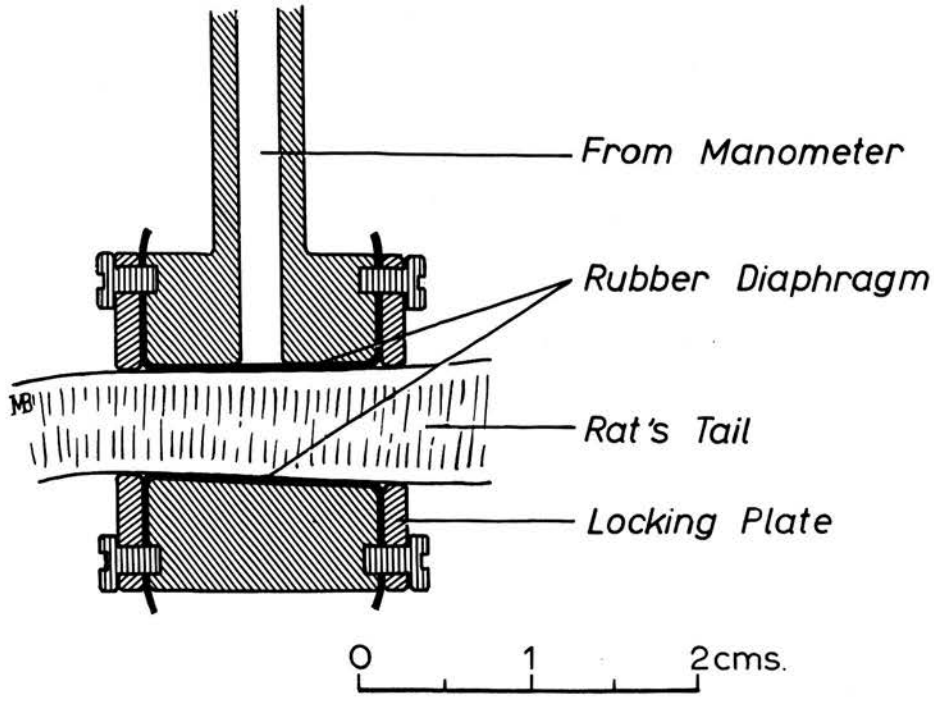

R
ATU
VOLUME
OFF
TUNER
OUTPUT
INPUT
C
L
G2
G1
V
B
WP
C.M
MB

Fig. 33.


From Manometer
Rubber Diaphragm
Rat's Tail
Locking Plate
MB
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1
2cms.

Fig. 34.

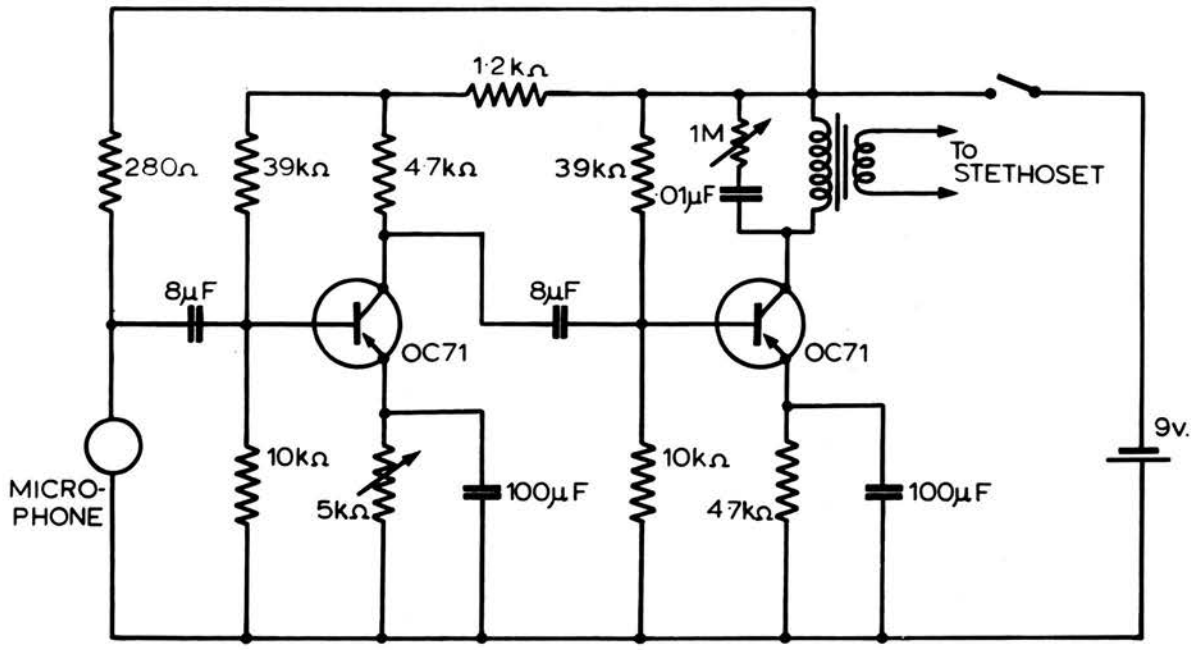

1·2kΩ
280Ω
39kΩ
4·7kΩ
39kΩ
1M
·01μF
To
STETHOSET
8μF
8μF
OC71
OC71
9v.
MICRO-
PHONE
10kΩ
5kΩ
100μF
10kΩ
4·7kΩ
100μF

Fig. 35.

Materials and Methods.

2. Measurement of Systolic Arterial Blood Pressure.

The method used was essentially that devised by GALLACHER & GRIMWOOD (1953) with some modifications.

Basically the apparatus consists of two parts (Fig. 33). The first part consists of a mercury manometer which both transmits and records pressure to a rubber cuff (Fig. 34), which lies around the rat's tail, i.e. a sphygmomanometer. The cuff was turned from a piece of solid brass and plates turned to fit over the ends. The rubber diaphragm was made from latex rubber by dipping a plaster of Paris replica of the brass cuff into liquid latex rubber (DUNLOP LATEX, A.330), and allowing the rubber to polymerise in air. The cuff was 2 cm. long and tapered slightly. This type of cuff was first described by BYROM & WILSON (1938).

The second part of the apparatus consists of the lever, microphone, amplifier and earpiece.

The lever transmits the pulsations of the caudal artery to the carbon microphone. The gimbals incorporated in the lever prevent transmission of the noise produced by tail movements caused by respiration. Respiration, the rate of which is approximately 100 per minute, causes the tail to move back and forward in its long axis. If the noise of this movement is allowed to be transmitted to the microphone, it muffles and obscures the pulse rate, which is approximately 300 per minute in the rat.

The/

The microphone is of the carbon type and of high sensitivity. A pulse in the caudal artery lifts the end of the lever resting on it, the lever rocks on the fulcrum at G2, and presses on the microphone. The increased pressure on the carbon granules produces a decrease in the internal resistance of the microphone, across which a voltage is applied from a 9 volt battery.

The change in current is magnified by the amplifier (Fig. 35) and transmitted to the ear microphone which is a deaf aid earpiece. A tuner is incorporated in the amplifier which cuts out high pitched noises. In practice, the pulse is heard as a very rapid tapping.

GALLACHER & GRIMWOOD (1953) used a microammeter to display the changes in current across the carbon microphone, and they did not include gimbals in their lever. The advantage of using gimbals has been explained above.

It was found in preliminary attempts in the present work that the innertia of the pointer in the microammeters used did not permit each individual pulse to be clearly seen, and for this reason an ear microphone was substituted.

Technique.

1. The rats must be warmed to produce vasodilatation in the tail before the pulse in the caudal artery can be heard. It cannot be heard under normal conditions of room temperature. This point has been emphasised/

emphasised by all previous workers and the effects of varying lengths of heating investigated by DODSON & MACKANESS (1957). They found that this was not critical and recommended placing the rat in a box kept at 40°C. for 20-30 minutes. In practice, it was found that placing an infra-red lamp over the rat cage for 10 minutes was the easiest method. To maintain the caudal vasodilatation, a platform (W.P.) was provided for the tail to lie on. This was kept at 42°C. by means of a small electric bulb and thermostat.

2. The rat was lightly anaesthetised with ether.

There has been considerable debate in the literature on the advisability of anaesthesia during blood pressure determination. It is quite feasible to measure blood pressure without anaesthesia, but training for the animals is required for consistent results to be obtained. Any struggling prevents accurate measurement. BYROM (1947) recommends ether anaesthesia, and suggested that light ether anaesthesia should depress blood pressure only slightly. DODSON & MACKANESS (1957) investigated the effect of varying levels of ether anaesthesia in the rat. They found that light anaesthesia had no detectable effect on blood pressure over a period of ten minutes. Deep anaesthesia lowered blood pressure considerably.

3./

3. The animal was then placed on the apparatus; its tail threaded through the cuff; and the lever adjusted, by means of the screw at G2, so that it lay lightly on the tail. The pulse could then be heard in the earpiece.

4. The pressure in the cuff was then raised to around 150 m.m. Hg. by means of the bulb (B) and valve (V) (Fig. 33). The pulse was then absent.

5. The pressure, as observed in the manometer, was lowered slowly until the noise of the pulse was heard.

6. The pressure was again raised by about 7 m.m. Hg. and lowered again very slowly so that the pressure at which the pulse was first heard could be accurately taken.

The measurement was repeated each time. In practice the two readings never varied by more than 2 m.m. Hg. which is one increment on the scale.

Materials/

Materials and Methods.

3. Conduct of the Experiment.

10 wistar rats, 5 male and 5 female of a line-bred strain maintained in the University of Edinburgh were used. They were four and a half months old, and siblings.

The males weighed from 305 gm. to 350 gm., mean 331 gm., and the females from 225 gm. to 255 gm., mean, 239 gm.

The normotensive systolic pressures were measured five times in each animal at daily intervals. These were taken at 5 a.m., 10 a.m., 3 p.m., 8 p.m. and 12 midnight.

Each animal was then injected intramuscularly in the hind leg with guanethidine, 10 mgm./kg.. The systolic pressure was then measured at 6 hourly intervals for 24 hours.

The animals were then left for four days to allow all effects of the drug to wear off, and the injection and measurements were repeated. This time blood pressure measurements were taken three hours after injection and every sixth hour after this.

By combining the two sets of systolic pressures, measurements were available for every third hour after injection.

After an interval of one week, the experiment was repeated on the same animals using hydralazine, at a dosage of 8 mgm./kg..

Results/

Results.

The individual figures for both normotensive systolic pressures and hypotensive pressures are in appendix 1.

Normotensive Systolic Pressure.

All rats. Mean = 125.5 m.m. Hg., S.D. = 10.3 m.m., n = 50.

5 male rats. Mean = 129.9 m.m. Hg., S.D. = 7.2 m.m., n = 25.

5 female rats. Mean = 121.0 m.m. Hg., S.D. = 12.8 m.m., n = 25.

The pooled mean depressions of systolic blood pressure and the range after injection of guanethidine and hydralazine in the ten rats are shown in Figs. 34 and 35.

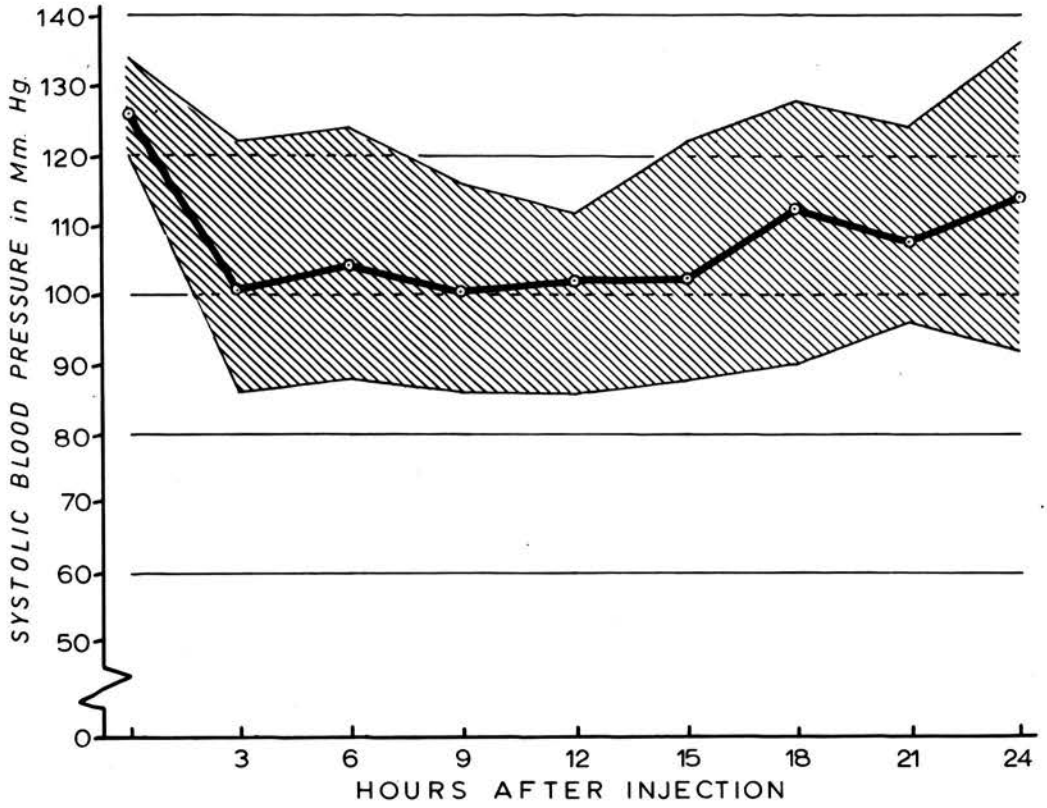
The pooled mean systolic pressure during the 24 hours after an injection of 10 mgm/kg. guanethidine was 105.6 m.m. Hg.. This is 84.1% of the pooled mean normotensive value of the same animals.

The pooled mean systolic pressure during 24 hours after an injection of 8 mgm/kg. hydralazine was 96.6 m.m. Hg.. This is 77.0% of the pooled mean normotensive value.

Subsequent to the injection of guanethidine, all the animals developed diarrhoea. This seemed to affect the male rats more than the females, as evidenced by the fluidity of the stools. This effect was much less obvious after 12 hours and had disappeared completely in 15 hours.

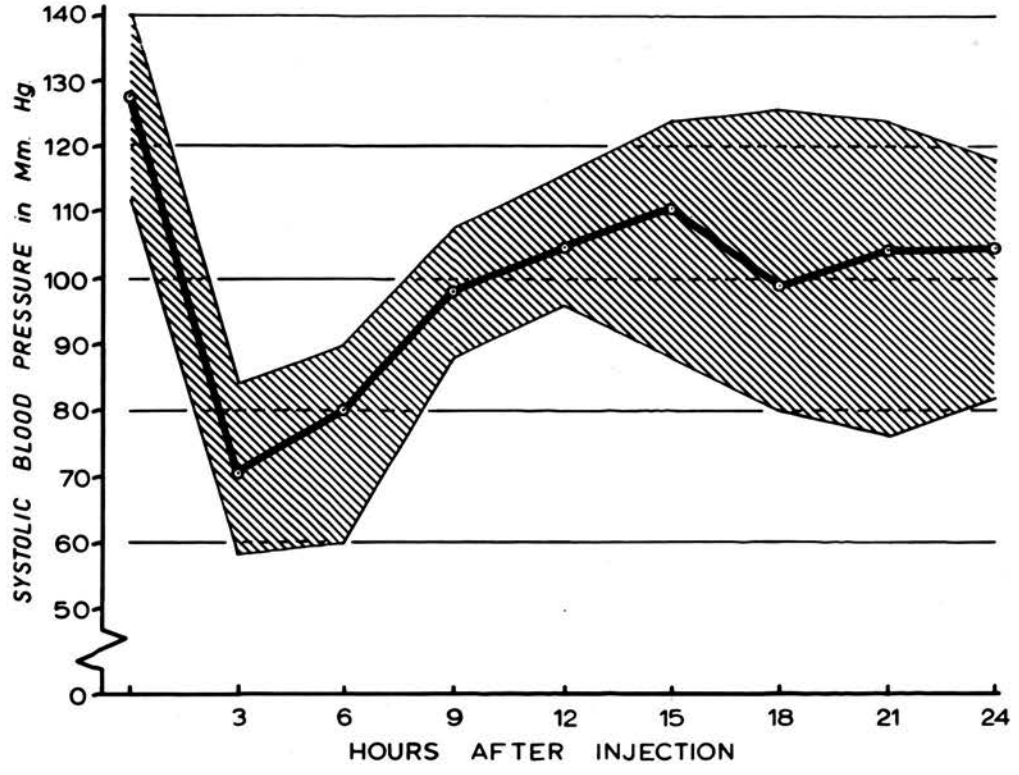
No other effect of either drug was observed. The rats remained healthy throughout and subsequent to the experiments.

Discussion/



GUANETHIDINE

Fig. 36.



HYDRALAZINE

Fig. 37.

Discussion.

The normotensive systolic pressures are within the usual range found in rats of this age and size, as are the standard deviations. ALEXANDER (1957) gives a mean of 125 m.m. Hg. for rats of around 250 gm. with a standard deviation of around 10 m.m., when no distinction between sexes is made.

The difference between the mean systolic pressures for males and females, 129.9 m.m. Hg. and 121.0 m.m. Hg., is significant, that is, the difference between the means differs by more than twice the value of the standard error of the difference.

$$\text{Difference} = 129.9 - 121.0 = 8.9$$

$$\text{Standard Error of Difference} = \sqrt{\frac{(SD_1)^2}{n_1} + \frac{(SD_2)^2}{n_2}} = \sqrt{\frac{7.2^2}{25} + \frac{11.2^2}{25}}$$

$$= \underline{2.7}$$

It seems probable that this difference is a reflection of the difference in weights between males and females rather than a sex difference. It has been shown (ALEXANDER, 1957, BYRON, 1947) that the mean systolic blood pressure of rats increases with increase in weight.

The variations in blood pressures found at different times of the day and night are not significantly different, using the same criterion for significance. It may be that diurnal variation in blood pressure in the/

the rat does exist but, if so, it is so slight as to be undetectable in the number of observations used in this experiment.

The results show that an average reduction of systolic arterial blood pressure of the order of 16% can be obtained by daily intramuscular injection of guanethidine at a dosage of 10 mgm/kg..

The diarrhoea which was observed to develop subsequent to guanethidine, lasting around 12 hours, is analagous to that found in dogs by MAXWELL et alia (1960). It has also been observed clinically in some patients taking the drug.

With a dosage of 8 mgm/kg. hydralazine, an average depression of systolic arterial pressure of 23% was obtained.

The results confirm what one could reasonably expect from knowledge of the actions of these drugs in other experimental animals and in man.

Conclusions.

It was concluded that, guanethidine and hydralazine were suitable drugs to reduce normotensive arterial blood pressures in the rat for the subsequent experiments on the eruption rate under hypotensive conditions.

Experiment II.

Introduction.

No information could be found in the literature on the action of hypotensive drugs on capillary pressures. For the subsequent experiment designed to observe any changes produced on eruption rate by administration of these drugs, it was necessary to find out what pressure changes were produced in the small vessels, if any. In the absence of such change, the subsequent experiment could not throw any light on the theory that eruptive pressure is derived from blood pressure in the small vessels.

Object: To measure the effects of guanethidine and hydralazine on the capillary and minute vessel pressure of the normotensive rat.

Method.

1. Drugs. The drugs were hydralazine and guanethidine administered in the same way as in the previous experiment.

2. Measurement of Capillary Pressure.

The only satisfactory method of measuring absolute intra-capillary pressure was devised by LANDIS (1930). He inserted a micropipette of glass with an aperture of about 10 microns into various parts of the capillary loops of the skin of the nail-bed of man and into the mesenteric capillaries of various animals. The pipette was moved by a micromanipulator and observed through a microscope. It was connected via

a/

a system of citrate containing tubes to a mercury manometer. When the pipette was inserted into a capillary, the pressure was adjusted until blood neither entered or left the pipette. This pressure was taken as the internal capillary pressure. LANDIS found a mean pressure in the arterial limbs of the capillary of 32 m.m. Hg., range 21 m.m. to 48 m.m., in the middle of the loop the mean pressure was 20 m.m. Hg. range 15 m.m. to 32 m.m., and at the venous end a mean of 12 m.m. Hg., range 6 m.m. to 18 m.m..

While admitting that this would have been the best technique to use, it was decided that the cost of the apparatus and the practice necessary to use it properly, were obstacles sufficiently substantial to make the use of a simpler technique desirable. It was considered that in this experiment absolute values for capillary pressure were not essential, as the object was to make a comparison of pressures in normotensive and hypotensive animals.

For these reasons, a much older method of measuring capillary pressure was used. This was based on the technique originally devised by ROY & BROWN (1879) for measuring capillary and small vessel pressure in the web and tongue of the frog.

The apparatus used was based on that described by DANZER & HOOKER (1920) but many modifications were introduced.

The apparatus is illustrated in Fig. 38 and Fig. 39, and can most easily be described by reference to these drawings.

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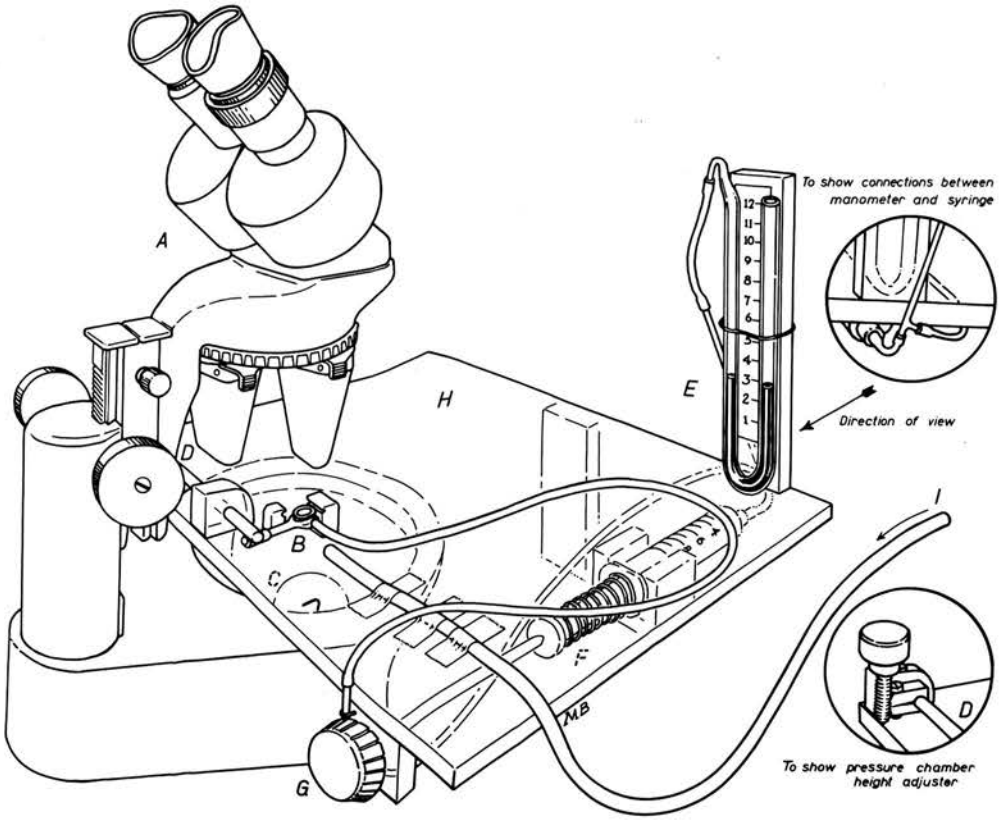

A
B
C
D
E
F
G
H
I
MB
To show connections between manometer and syringe
Direction of view
To show pressure chamber height adjuster

Fig. 38.

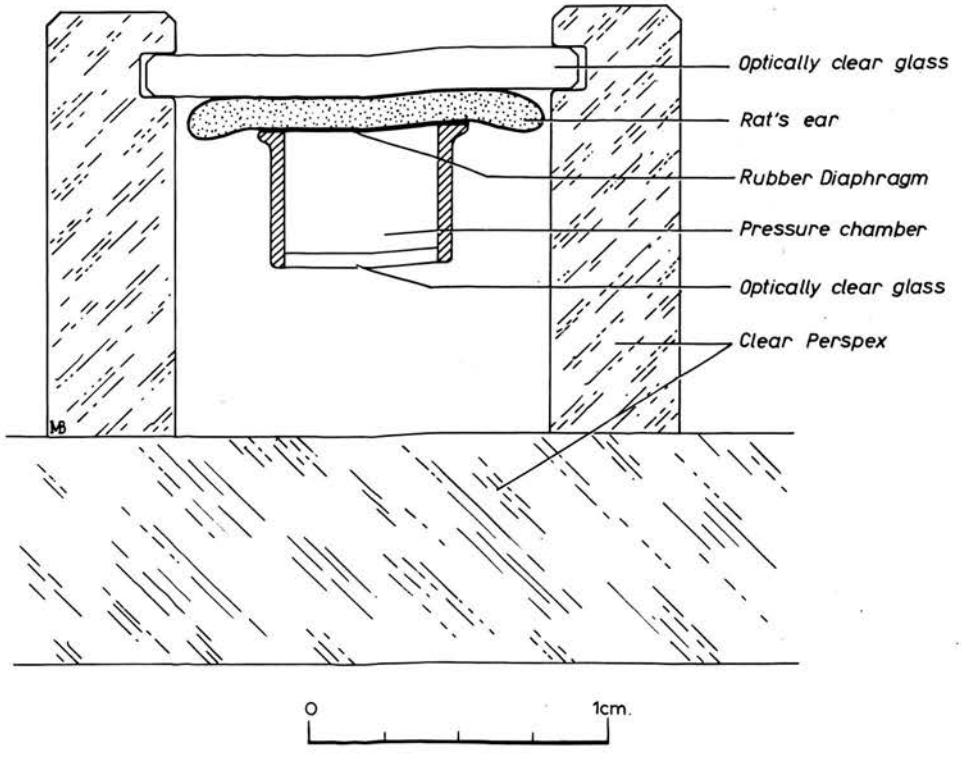

Optically clear glass
Rat's ear
Rubber Diaphragm
Pressure chamber
Optically clear glass
Clear Perspex
MB
0
1cm.

Fig. 39.

The pressure chamber (Fig. 39) was made from a brass cylinder 5 m.m. in diameter, one end of which was sealed by cementing into it a piece of optically clear glass, turned on a lathe to fit it exactly. The other end of the cylinder had a slight lip around the free edge. To this lip a piece of thin, transparent latex rubber was attached by an adhesive, EASTMAN 910. The rubber was not stretched and was cut from a piece of sheet latex which was not flat, but slightly domed, with the convexity of the dome outwards. The chamber was airtight.

The pressure chamber was mounted on gimbals so that it could rotate in any direction about a point at its centre, but the centre was held at a constant height which could be adjusted by means of the screw shown in the lower inset drawing.

The pressure chamber was connected by small bore polythene tubing to a mercury manometer (E), by means of which the air pressure in the chamber could be altered. The manometer was controlled by a 10 ml. glass syringe which was made airtight by lubricating the plunger with Vaseline. The airway connections between manometer, syringe and pressure chamber are shown in the upper inset drawing.

So that fine movements of the syringe plunger could be easily made, the syringe was mounted on the lower surface of the perspex base of the apparatus, the plunger was spring-loaded, and depression of the plunger was achieved by use of the coarse screw (G).

The perspex base of the apparatus lay on the stage of a stereoscopic microscope. Illumination was provided from a 12 volt. car headlamp bulb below the perspex base.

Technique/

Technique.

1. The rat was anaesthetised by intraperitoneal barbiturate. It was found that the most convenient anaesthetic from the point of view of length of induction and length of anaesthetic was a combination of methohexitone sodium and pentobarbitone sodium in dosages of 0.6 mgm./kg. and 6 mgm./kg. respectively. This combination was found to give an induction period of a few minutes and an anaesthetic period of 20-30 minutes which were adequate for the purposes of this experiment.

2. Once anaesthetised, the rat's ear was thoroughly washed with soap and water, and dried. Usually the left ear was used, but either ear could be chosen.

The outer surface of the ear was then smeared with an aqueous gel of carragheen moss in which the viscosity characteristics had been modified by addition of propylene glycol and glycerine. This gel is manufactured commercially and sold by London Rubber Industries Ltd..

The gel was used in preference to oil, which had been used by previous workers, as it was found in preliminary experiments that oil gave rise to a loss of elasticity and breakdown of the rubber top of the pressure chamber. This necessitated replacing the diaphragm fairly frequently, and occasionally the diaphragm gave way during a series of measurements which meant that the series had to be repeated.

The use of the carragheen, propylene glycol, glycerine gel maintained the rubber diaphragm in good condition, and throughout this experiment the same diaphragm was used: no leakage developed.

The/

The gel performed the function of enabling visualisation of the capillaries through the microscope as well as immersion oil.

3. The rat was then laid on its side on the perspex platform (H) and one ear laid between the rigid piece of glass and the pressure chamber. By means of the screw adjustment shown in the lower inset in Fig. 38, the chamber was raised until it held the ear against the glass plate without crushing it or obstructing the blood supply. The gimbals were necessary for this as the rat's ear is not uniform in thickness.

4. The light was then turned on and air blown gently through the large tube from a reservoir of compressed air to prevent overheating of the ear by the light source. The capillaries were then visualised through the stereoscopic microscope, at a magnification of X140 or X100. The flow of blood through the small vessels could be seen clearly. It was found that a stereoscopic microscope was preferable as it gave a greater depth of focus, enabling the observer to see more vessels in one field.

5. The entire area of ear under the pressure chamber was then scanned rapidly to make sure that no obstruction to the normal blood flow was present. If necessary the height of the pressure chamber was adjusted.

The attention was then focused on one capillary and, using the screw (G), the pressure in the chamber was raised. This pressure was transmitted to the ear by the elastic diaphragm, and it is assumed that little or no pressure was taken up in distorting the diaphragm itself because of the curved surface of the rubber.

When/

When the diaphragm was brought up against the ear, the domed surface wrinkled and these wrinkles slightly obscured the view of some capillaries. The shadows cast by the wrinkles disappeared when the pressure in the chamber was raised by a few millimetres of mercury.

The pressure in the chamber was raised until blood flow completely stopped in the capillary under observation. The pressure was then slowly lowered and the following sequence of events could be observed:-

(a) The blood flow resumed in the opposite direction to what it had previously been.

(b) At a lower pressure, the retrograde blood flow ceased and a to and fro motion of the corpuscles could be observed.

(c) At a still lower pressure, a very slow flow was observed in the original direction.

(d) At a lower pressure, and quite suddenly, rapid flow was re-established in the original direction.

This sequence of events was almost invariably seen. It has been described by DANZER & HOOKER (1920) as occurring in man under similar experimental conditions. DANZER & HOOKER (1920) suggested that the pressure at (d) in the list above should be adopted conventionally as the capillary pressure, and all measurements in this series were taken at this point.

With/

With practice, repeatable measurements can be made by this method, but the best confirmation of the reliability of the method is given by the control figures obtained from the animals used in the experiment.

The actual pressures obtained by this method are almost invariably higher than those obtained by the direct method of measurement. LANDIS (1930b) found an average capillary pressure of 22 m.m. Hg. in the rat mesentery, in a series of 36 measurements in an unstated number of decerebrate animals. In anaesthetised guinea pigs, he found an average capillary pressure of 29 m.m. Hg.. In view of this large difference between two similar animals, it would seem that the low figures LANDIS obtained on rats were attributable, at least in part, to the fact that the animals were decerebrate. No other figures for capillary pressures in rats could be obtained in the literature.

Capillary Circulation in the Rat Ear.

CHAMBERS & ZWEIFACH (1944) demonstrated that the capillary circulation in the rat was considerably more elaborate than had been thought previously. To briefly state their findings, they showed that blood flowed from the arteriole into a vessel of capillary dimension but which had discontinuously muscular walls. This they called the metarteriole. This vessel ran directly from the arteriole to the venule, as a thoroughfare channel but lost its muscle fibres, approximately half way along. From the metarteriole, true capillaries branched off, a sphincter being found at the point of origin. The venular half of the thoroughfare channel had capillaries feeding blood back into it.

Elsewhere/

Elsewhere true capillaries arose directly from the arteriole, again with a sphincter at the origin.

CHAMBERS & ZWEIFACH showed that the metarteriole reacted to local changes in the tissue fluids, such as the introduction of epinephrine or histamine, and to nervous stimuli, in contra-distinction to true capillaries which did not react.

They demonstrated the presence of the matarteriole - through fare channel complex in various anatomical situations such as the omentum and tongue of the frog, mesoappendix, interdigital web and scrotum of the rat and in the omentum of the dog, and in various situations in the mouse. They suggested that this complex existed in practically all anatomical sites in mammals and this is now accepted as a generalisation, various other tissues having been shown to conform to this pattern, for example the dental pulp (PROVENZA, 1958).

It would seem reasonable to assume that this pattern of capillary circulation is present in the rat ear, although there is no direct evidence in favour of its existence.

In the direct microscopic observation of the minute vessels of the rat ear used in this experiment, it was not possible to distinguish between metarterioles, non-muscular thoroughfare channels, and true capillaries. The method, then, is more accurately described as measuring minute vessel blood pressure. However, for the sake of brevity, the method has been described in this work as measuring capillary pressure.

Arterioles/

Arterioles could be distinguished from other components by the pulsatile nature of the blood flow through them. They were also tortuous and blood flow in them was more rapid, giving rise to continual minute flashes as the leucocytes refracted the transmitted light. These flashes were also noticeable in the other small vessels, but to a lesser extent. Venules could be distinguished by their straighter course, by a slightly darker colour and by the almost total absence of flashes due to a considerably slower rate of flow which could be seen on close observation.

No observations have been published on the difference in pressure between metarterioles, non-muscular thoroughfare channels and true capillaries.

Materials.

Twelve Wistar rats, six male and six female, of the strain kept in the University of Edinburgh were used. They were siblings and aged 3½ months at the beginning of the experiment.

Normotensive capillary pressures were taken for each rat, the pressure in approximately thirty different capillaries being measured on each animal.

One week later the animals were injected intramuscularly with hydralazine at a dosage of 15 mgm./kg. at 10 a.m.. The capillary pressure measurements were then repeated on each animal between 2 p.m. and 5 p.m. on the same day.

One/

One week later, with no intervening drug therapy, the capillary pressures were taken again on the same animals.

One week later, the same animals were injected intramuscularly with guanethidine in doses of 10 mgm./kg. at 10 a.m. and the capillary measurements repeated the same day between 2 p.m. and 5 p.m..

Results.

One animal died due to an accidental overdose of anaesthetic while the pressure after hydralazine was being measured. Therefore, all results from this animal have been omitted. The other animals remained healthy throughout the period of the experiment.

The individual figures obtained from each animal are in appendix 2A, where they have been arranged in ascending order.

The pooled mean values obtained are as follows:-

Mean Capillary Pressure during 1st. normotensive series
- 30.6 m.m. Hg., S.D. 7.5.

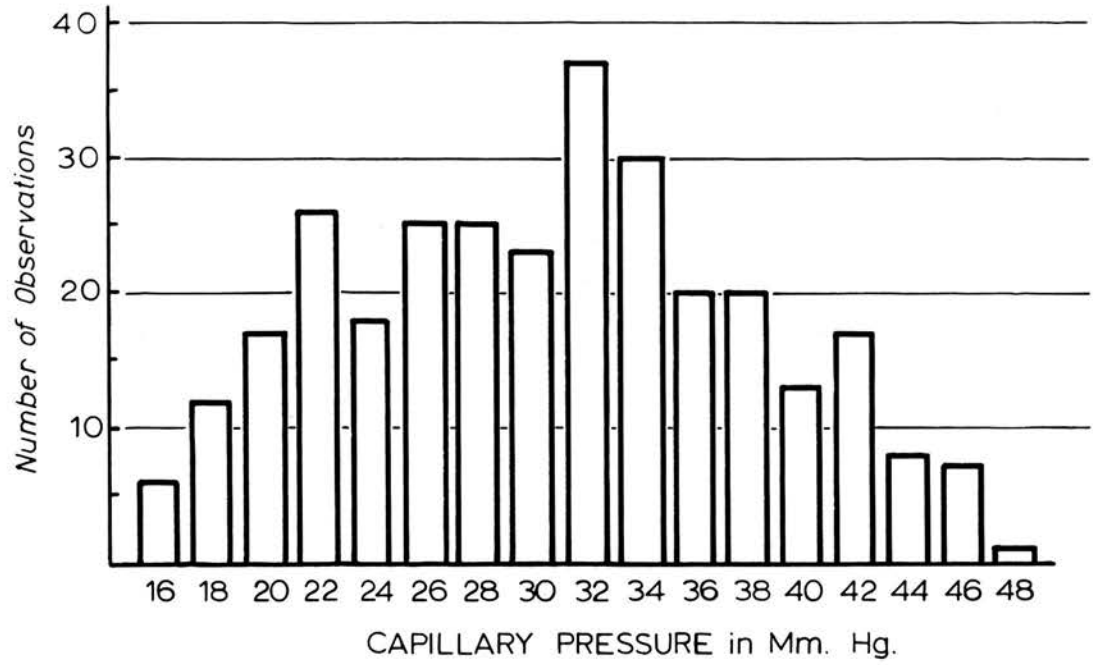
Mean Capillary Pressure after hydralazine
- 37.1 m.m. Hg., S.D. 8.4

Mean Capillary Pressure during 2nd. normotensive series
- 30.4 m.m. Hg., S.D. 7.3.

Mean Capillary Pressure after guanethidine
- 36.2 m.m. Hg., S.D. 7.4

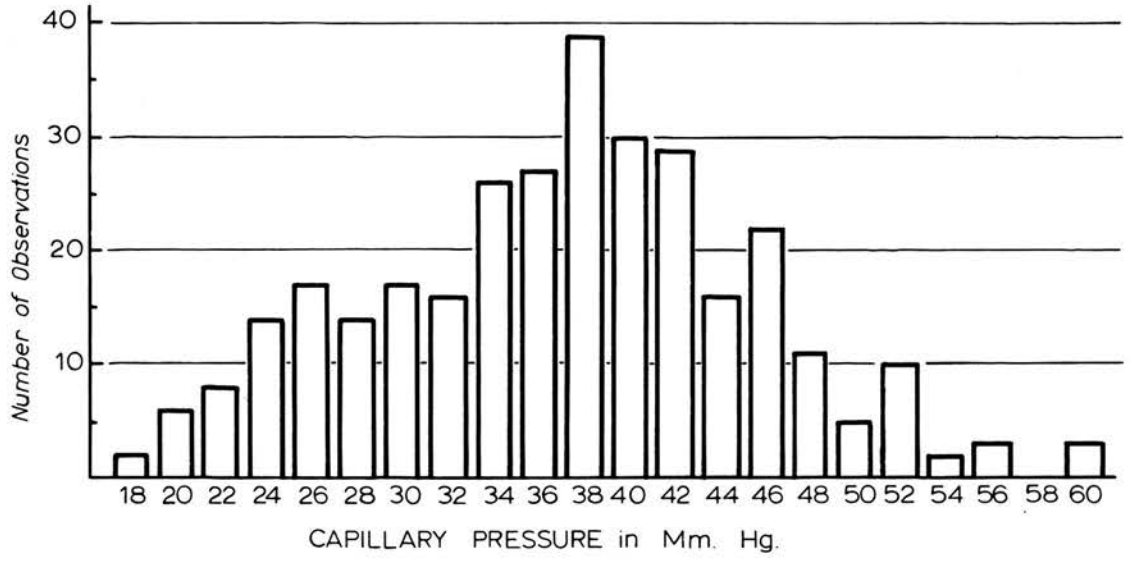
The individual means for each animal are given in the appendix.

A/



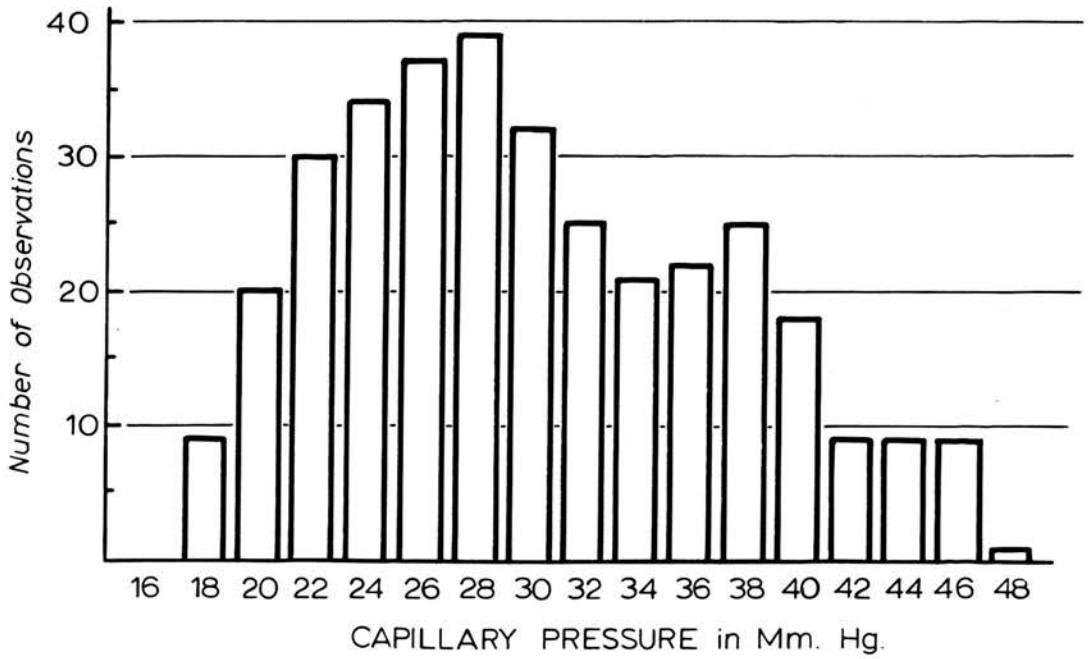
1st CONTROL

Fig. 40.



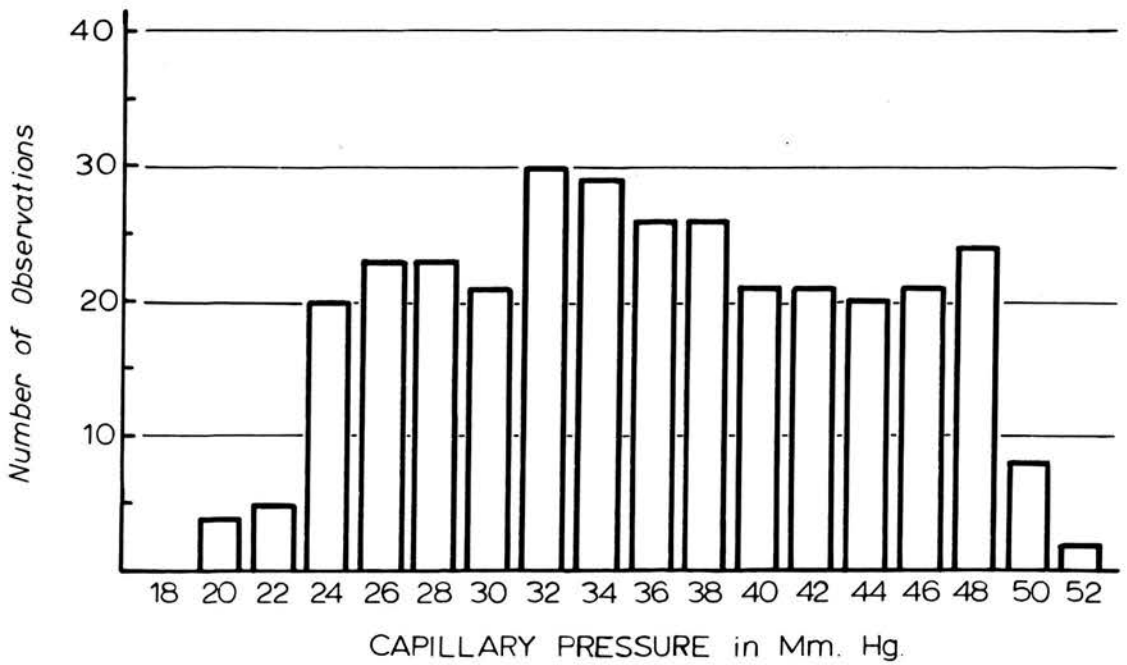
HYDRALAZINE

Fig. 41.



2nd CONTROL

Fig.42.



GUANETHIDINE

Fig. 43.

A definite impression was obtained that the capillaries were more easily seen subsequent to the injection of the hypotensive drugs. It was difficult to decide whether this was because the vessels were wider than under normotensive conditions or because more vessels were patent in any one microscope field. No count of total numbers of vessels patent in unit area under normotensive and hypotensive conditions were made, but additional capillaries could be seen to open and come into functional use as the ear was warmed by the light source. If the cooling air stream was switched off, still more capillaries could be seen to open up. It had been found in preliminary work, that the air stream was necessary to prevent burning of the ear.

Analysis of the Results.

Type of Distribution - The first analysis was to ascertain whether or not the observations formed a normal distribution, taking into account the error arising from sampling.

The simplest way to assess this is to construct histograms of the frequency distributions of each series. This was done and the histograms are shown in Figs. 40 - 43. It will be seen that none of them approximate closely to the appearance of a normal distribution, with the possible exception of the observations after the injection of hydralazine. In the others, there is an absence of the peaking around the mean, characteristic of the normal distribution; instead each histogram has a rather flat-topped appearance.

To/

To gain further information on how the distributions of the observations differed from normal distributions, a goodness of fit test was applied to each series, that is the expected frequency distribution was calculated from the general formula for a normal distribution, and the expected frequency was compared to the observed frequency using chi-square to assess the departure from normality.

In practice, the expected frequency distribution was derived from the Biometrika Tables for Statisticians (PEARSON & HARTLEY, 1954) Table 1.

In deriving the expected frequencies, the values for means and standard deviation were halved, as the observations were made originally on a simple U-tube mercury manometer where readings were made to an accuracy of 1 m.m. Hg. and then doubled. It was considered appropriate to construct a normal distribution curve for comparison with the actual manometer readings, and not the doubled values.

The goodness of fit tests and the chi-square value for each series are in appendix 2B.

The values obtained for chi-square were:-

1. First normotensive series - 22.18, d.f. 13.
2. After hydralazine - 21.73, d.f. 15.
3. Second normotensive series - 37.26, d.f. 13.
4. After guanethidine - 60.93, d.f. 14.

Thus/

Thus all chi-square values were high, and, although the first two above are just less than the five percent point for chi-square with the requisite degrees of freedom, it can be concluded that these observations are not normally distributed.

Significance of Differences Between Means.

Reference to the histograms shows that the distribution of observation in each series is broadly similar, that is, the histograms are flat-topped with short tails towards the lower values and longer tails towards the higher values.

Tests for significance of difference between the means of similarly distributed samples do not differ greatly from similar tests between means of normally distributed samples, although the confidence limits for each mean are usually greater than is the case with means of normal distributions.

Therefore tests for significance of difference between the mean normotensive pressures and the mean pressures found after the injection of the drgus were applied as follows:-

1. Between the two normotensive series:-

Normotensive 1.		Normotensive 11.	
Mean	30.63 m.m. Hg.	Mean	30.38 m.m. Hg.
S.D.	7.56	S.D.	7.28
n	305	n	340

Difference between means $(\bar{x}_1 - \bar{x}_2) = 0.25$

Standard Error of Difference $(S\,\bar{x}_1 - \bar{x}_2)$

$$= \sqrt{\frac{7.56^2}{305} + \frac{7.28^2}{340}}$$

$$= 0.591$$

Therefore there is no significant difference between the means of the two normotensive series.

2. Between the first normotensive series and the mean of the observation after injection of hydralazine.

Normotensive 1.		After Hydralazine.	
Mean	30.63 m.m. Hg.	Mean	37.07 m.m. Hg.
S.D.	7.56	S.D.	8.44
n	305	n	317

Difference between means $(\bar{x}_1 - \bar{x}_2)$ = 6.44.

Standard Error of Difference

$$(S\,\bar{x}_1 - \bar{x}_2) = \sqrt{\frac{7.56^2}{305} + \frac{8.44^2}{317}}$$

$$= \underline{0.64.}$$

Now $$t = \frac{x_1 - x_2}{S_{\bar{x}_1 - \bar{x}_2}}$$

Substituting the above figures, we have

$$t = \frac{6.44}{0.64} = \underline{10.05}.$$

Degrees of Freedom $= (n_1 - 1) + (n_2 - 1)$

$= 304 + 316$

$= 620.$

From the tables we have for t = 10.05 with 620 degrees of freedom, p. $<$ 001. In other words, the difference is highly significant, as the odds against the difference being due to chance are greater than 1,000 to 1.

3./

3. Between the mean of the second normotensive series and the mean of the observations after injection of guanethidine.

Normotensive 11.		After Guanethidine.	
Mean	30.38 m.m. Hg.	Mean	36.20 m.m. Hg.
S.D.	7.28	S.D.	7.40
n	340	n	325

Difference between means $(\bar{x}_1 - \bar{x}_2)$ = 5.82 m.m. Hg.

Standard Error of Difference = $\sqrt{\frac{7.28^2}{340} + \frac{7.40^2}{325}}$

= 0.57.

$$t = \frac{\bar{x}_1 - \bar{x}_2}{S_{\bar{x}_1 - \bar{x}_2}}$$

Substituting, we have $t = \frac{5.82}{0.57}$

$= \underline{10.21}$

Degrees of Freedom = $(n_1 - 1) + (n_2 - 1)$

= 663.

From the t tables, we find for t = 10.21 and 663 degrees of freedom, $p < .001$.

In other words, this too is a highly significant difference.

It/

It can be concluded that, in view of the similarities of the frequency distributions being compared, and as the t - values are so high, that the differences between the means under normotensive and hypotensive conditions are significant. The effect of guanethidine and hydralazine in the dosages used is to raise the mean capillary pressure by around 20%.

Similar tests for significance of difference between the pooled normotensive means for male and female animals were carried out. These showed no significant difference between males and females.

Analysis of Variance.

To provide more information on where the differences lay between capillary pressures under normotensive and hypotensive conditions, an analysis of variance was carried out in the following manner.

As there were roughly 30 observations made at each capillary pressure estimation on each rat on each of four occasions, these were laid out in ascending order and divided into three groups with roughly 10 observations in each. The actual figures are shown in appendix No. 2C. Where the number of observations was not exactly thirty, the observations were divided into three groups as nearly as possible.

Let us consider now the analysis of variance between the first normotensive series of observations and the observations after injection of hydralazine. This will serve to describe all three of the analyses carried out.

The/

The individual differences between each normotensive pressure and each hypotensive pressure were calculated and laid out in column D1. Then the mean and standard deviation of the sum of the differences for each block of ten observations were calculated. Each of these steps was carried out on each animal individually.

A table was then set out for the analysis of variance as shown below. For the same reason as was given for the construction of the expected normal frequency distribution for these observations, it was considered appropriate to halve the mean differences. This, in fact, does not affect the variance ratio which is the same whether the differences are halved or not.

The analysis of variance was then carried out by the method described by BAILEY (1959).

The same procedure was then repeated so that analysis of variance could be carried out between the two normotensive series and between the second normotensive series and guanethidine.

In essence, this method enables a comparison to be made between the effects of each drug on each animal (animal variation) and also the effects of the drugs on each of the three pressure ranges, i.e. low, middle and high (block variation).

The general tables of data for these analysis are set out in appendix No. 2D, only the derived analyses of variance being given here.

Analysis/

Analysis of Variance Between the Two Normotensive Series of Observation.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio
Animals	35.0534	10	3.5053	6.18
Blocks	3.3033	2	1.6515	2.91
Residual	11.3434	20	0.5672	
Total:	49.7001	32		

The one per cent point of variance ratio distribution where $f_1 = 10$, $f_2 = 20$, is 3.37, where $f_1 = 2$, $f_2 = 20$, the five per cent point is 3.49. Therefore in this case there is a highly significant variation between animals, but not between blocks.

Analysis of Variance Between First Normotensive Observations and the Observations After Injection of Hydralazine.

Sources of Variation	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio
Animals	119.2300	10	11.9230	13.29
Blocks	3.3195	2	1.6597	1.85
Residual	17.9465	20	0.8973	
Total:	140.4950	32		

Comparison with points of variance ratio distributions for the requisite degrees of freedom shows again that the variation between animals is highly significant, but not between blocks.

Analysis/

Analysis of Variance Between Second Normotensive Observations and the Observations after Injection of Guanethidine.

Sources of Variation	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio
Animals	14.2208	10	1.4221	1.76
Blocks	7.1686	2	3.5843	4.42
Residual	16.2047	20	0.8102	
Total:	37.5941	32		

In this case, comparison with the variance ratio distribution tables, shows that the variation between animals is not significant but that the variation between blocks is significant at the five per cent level.

Discussion.

It is not suggested that the values obtained for capillary pressures by this method were absolute. An immeasurable proportion of the applied pressure must have been dissipated in distorting and compressing the tissues and in causing movement of the extracellular fluid in the tissues. However, it seems reasonable to assume that this proportion of dissipated pressure would remain nearly constant for both normotensive and hypotensive animals and that comparison of the observations under the different conditions is not unreasonable.

It/

It was not surprising to find that the observations did not approximate closely to a normal distribution. It is known that capillary pressures fall throughout the length of the capillary (LANDIS, 1930a) so that the measurements were of data which fell into a range of values in each animal, not, as is commonly the case in biological measurements, of data which had one value for each animal with biological variation between animals, and between observations on the same animal at different times.

The frequency distribution observed was indeed much what one would expect from capillary pressure observations, that is to say, a flat topped curve with a short tail for the lower values. The longer tail found for higher values is perhaps explained by CHAMBERS & ZWEIFACH's (1944) finding of a few capillaries, which ran directly from arteriole to venule, and of the metarteriole. Presumably in both these vessels, the end closest to the arteriole has a fairly high pressure, approximate to that of the arteriole, though presumably modified by the sphincter at the point of origin.

As was explained in the analysis of the results, tests for significance of difference of means of similarly distributed frequency distributions do not differ greatly from similar tests between normally distributed distributions, although the confidence limits may be somewhat greater. In view of the high t values obtained, it seems safe to conclude that the differences found were significant, the main object of the experiment.

The/

The analysis of variance revealed some interesting data. Firstly in regard to the analysis between the two normotensive series of observations, animal variation is seen to occur, as one would expect in measurements of this type made on the same animals with a time interval of fourteen days. It can also be seen that there is no significant variation between the arbitrarily chosen pressure ranges.

These findings provide strong evidence in favour of the reliability of the method, as of course, does the fact that the means of the two normotensive series do not differ significantly, and are, in fact, reasonably close to one another.

The variance analysis between the first normotensive series and hydralazine shows that individual variance between animals was very marked. This can also be seen by examination of the individual means and standard deviations of differences laid out fully in appendix No. 2D. Examination of the general table of data for this group shows that three animals were only slightly affected by hydralazine, animals numbers 3, 7 and 10 and that animal number 9 was only slightly more affected. The remaining animals were affected to a very much greater extent.

The explanation of this finding of great animal variation is not obvious, but it may be put down to individual idiosyncracy to the drug, a form of words commonly used to cloak ignorance in this subject. It was not a noticeable effect when arterial blood pressures were measured subsequent to hydralazine by injection. It has been generally assumed previously, and, it is suggested, proved in this work, that the hypotensive effect of these drugs is produced by a decreased generalised vasomotor tone in the arteries/

arteries, arterioles and metarterioles, resulting in a decreased arterial blood pressure, the energy of the pressure produced by the heart beat being dissipated to a greater degree than normally in the capillary network. Whether this loss of vasomotor tone was equally distributed between artery, arteriole and metarteriole is a question which has never been answered.

As was explained in the introduction, the mode of action of hydralazine is obscure but it appears to have a central component as it has been shown to diminish the outflow of sympathetic, vasomotor impulses from the mid-brain (CRAVER et alia, 1951).

It may be that differential effects on the three types of arterial vessel can account for the animal variation, but this, of course, is mere speculation.

It should further be noted that the variation between blocks, i.e. low, middle and high observations, was not significant. This indicated that the entire range of capillary pressures were equally affected by the drug.

When the analysis of variance between the second normotensive series and the observations subsequent to injection of guanethidine is examined, the exact opposite state of affairs is found.

Firstly, the variation between animals is not significant, in other words the drug affected all the animals to approximately the same degree, which is in sharp contrast to the hydralazine group.

Secondly/

Secondly the variation between the blocks is significant. Examination of the full table of data in the appendix shows that this block variation is the result of an increased frequency of observations in the 40 – 50 m.m. Hg. range, particularly in the higher values. This is also shown in the histogram of the guanethidine frequency distribution and its effects are shown on the goodness of fit to a normal distribution test by the very high value of chi-square.

Again no clear-cut explanation of this finding can be advanced . However, a hypothesis to explain it, in relation to the findings of the effects of hydralazine on capillary pressure will be suggested. It is based on the assumption that these drugs affect the arterial vessels at different levels. It must be freely admitted that there is no direct evidence in favour of this assumption. If, however, one assumes that hydralazine affects chiefly the arteries and arterioles, while affecting the metarterioles not at all or to a much lesser extent, then it follows that blood must be reaching the arteriolar end of the metarterioles and of those capillaries coming directly from the arterioles at a higher pressure than under normotensive conditions. There would then be a much greater range of pressures in the capillaries than normally, but the pressures would fall steadily throughout this range. This conclusion coincides with the observations made of capillary pressure after the injection of hydralazine.

Again/

Again, if the assumption is made that guanethidine affects chiefly the metarterioles, while affecting the arteries and arterioles to a much lesser extent, then the observations made after this drug can also be explained. One consequence of metarteriolar dilatation would be that the range of pressures observed would not be markedly increased - as was found. However, a greater proportion of the dissipation of pressure would be transferred to the capillaries, which would mean that there would be many more capillaries with increased pressures. And, as was explained in the introduction, doubtless the pressure in many metarterioles themselves was measured, as these cannot be distinguished from true capillaries by this method. This perhaps explains the high numbers of observation made in the high 40 - 50 m.m. range.

Another possibility is that the difference found in the actions of guanethidine and hydralazine is the result of these drugs interacting in different ways with the barbiturate used to anaesthetise the rats.

Conclusion.

It should be emphasised that this experiment was not designed to provide information on the mode of action of the drugs used. The fact that the observations made on capillary pressure suggested a hypothesis on their mode of action was entirely serendipitous.

It has been shown that guanethidine and hydralazine, in the dosages used, both raised capillary pressure in the rat by around twenty per cent and were therefore suitable drugs to use in the subsequent experiments on the rate of eruption of the rat incisors.

Experiment III.

Object: To measure the effects on the rate of eruption of the rat incisor of the administration of hydralazine and guanethidine.

Materials and Method.

Method of Measuring Eruption Rate.

The method used was essentially that described by BRYER (1957). The procedure was as follows: (Fig. 44)

1. The rat was anaesthetised with ether.
2. When anaesthetised, one mandibular incisor was cut back out of occlusion by about 3 or 4 m.m. using a dental engine and a sharp diamond disc. A mark was made on the labial enamel of the other incisor as close as possible to the level of the cut surface of its neighbour.

A simple mask, made of acrylic, was placed over the rat's head during this procedure. The mask had a shelf as a mouth prop which, being inserted between the upper and lower incisors, steadied the incisors during cutting and prevented traumatisation of the soft tissues. By having a sponge kept damp with ether in it, the mask acted both as an open anaesthetic mask and as a mouth prop.

3. The animal was then placed in the holder, as shown in Fig. 44. This holder was a simple box, the lid of which was in two adjustable halves with a semi-circular hole in each half, lined with sponge rubber to hold the rat's neck.

It/

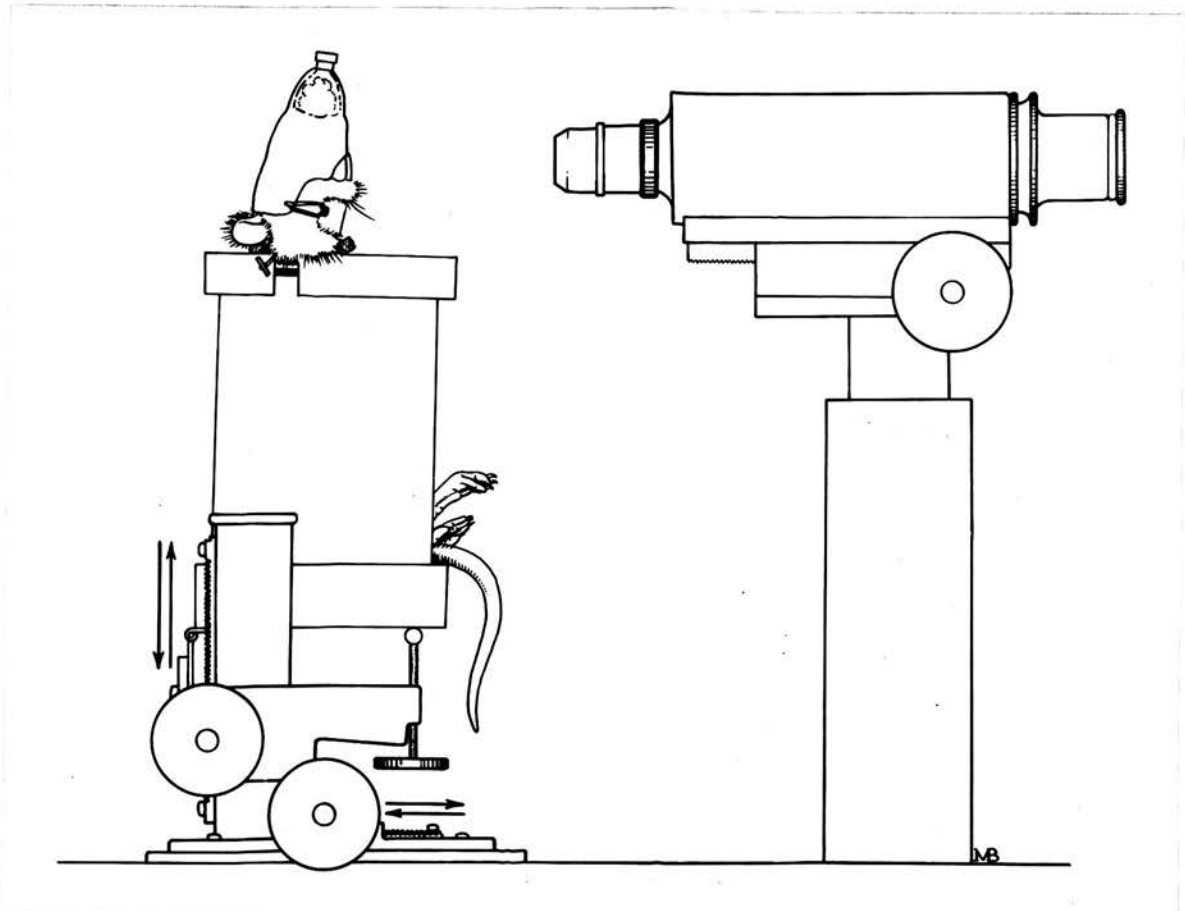


Fig. 44.

It was usually necessary for an assistant to hold back the lower lip so that the mandibular incisors could be seen. In many cases also, the assistant had to steady the head, which tended to nod in time with respiration. This was particularly noticeable with male animals.

4. The incisors were then viewed through the microscope which had a squared graticule in the eyepiece.

By means of the extension tube the microscope was adjusted so that each square on the graticule had a side of 0.1 m.m.. This meant that the measurements were made to an accuracy of plus or minus 0.05 m.m..

The height of the box could be altered by means of the racking adjustment shown in the diagram. The angulation of the box could be altered by means of the screw on which the front of the box rested. Both these adjustments were usually required to bring the incisors into view in a plane at right angles to the microscope. The height was corrected first and then the angle was assessed by observing the changing apparent length of the incisor as the screw was turned. When the incisor reached its maximum length, it was assumed that it lay at right angles to the microscope.

5. The measurements shown in Fig. 4 were then made, i.e. X_1 and Y_1. Forty- eight hours later the measurements were repeated, giving X_2 and Y_2. The incisor was then again cut back, a new mark made at the level of the new cut, and measurements X_1 and Y_1 retaken.

From these figures the rate of eruption of both cut and uncut mandibular incisors could be deduced.

Materials/

Materials.

Forty, four month old, Wistar rats, twenty of each sex, of the strain maintained in the University of Edinburgh small animal breeding station, were used. These animals were of a line bred strain and as few litters as possible were used to make up the totals.

They were caged in groups of five by sex, and were fed on stock rat cake and water ad libitum. In addition they were given whole meal brown bread.

They were divided into three groups, A, B & C. All animals had their rate of mandibular incisor eruption measured in the manner described every 48 hours over a total period of thirty-two days, making sixteen measurements in all for each animal.

Group A were given intramuscular injections of 0.2 mls. sterile normal saline from the tenth to the twenty-first day of the experiment.

From the tenth to the twenty-first day, inclusive, group B received 10 mgm./Kg. guanethidine intramuscularly in the hind leg daily.

From the tenth to the thirteenth day, inclusive, group C were given hydralazine in daily dosages of 4 mgm./kg.. From the fourteenth to the seventeenth day, the dosage of hydralazine to this group was 8 mgm./kg., and from the eighteenth to twenty-first day, the dosage was increased to 16 mgm./kg..

These drugs were made up in such concentrations that the volume being injected fell between 0.15 mls. and 0.25 mls. daily.

The/

The rats were weighed on each occasion on which the rate of eruption was taken. The weighing was carried out on a simple BUTCHART balance with lever arm and counterpoise for speed, and the weight taken to the nearest 5 gms..

Results.

Thirteen animals died during the course of the experiment. The cause of death in all cases was asphyxia due to aspiration of mucus. Occasionally it was possible to resuscitate a rat subsequent to obstruction of the trachea by mucus but generally this failed. The method of resuscitation used was to aspirate the mucus from the trachea by means of a fine pipette and rubber bulb and to apply manual artificial respiration.

Of the animals which died, nine died so early in the experiment that the results obtained from them were of no value. Accordingly, the observations made on these animals have not been included in the results.

The individual measurements of rate of eruption for each animal are given in appendix No. 3, A. Occasionally the uncut incisor was found to have fractured, so that no measurement was possible.

Blanks occurring in the table are due to tracheal obstruction during the measurements, and although resuscitative measures were successful, the observations were not completed as the animal recovered consciousness and it was considered inadviseable to reanaesthetise.

With/

With the exception of those animals which were successfully treated for tracheal obstruction, the rats remained healthy throughout the time of the experiment. When receiving guanethidine most animals had semi fluid faeces, but no other effects of the drug were noticed.

Usually, but not invariably, the animals which had had a tracheal obstruction exhibited a general malaise for the next 24 - 48 hours. This was shown by a roughening of the fur and a tendency to "sniffles", indicative of upper respiratory inflammation. The signs did not persist for more than 2 days in any case.

The observations on weight of each animal are given in appendix No. 3,B. A number of weights are missing and the reason for these omissions is that the rat recovered consciousness prior to the weighing being completed. It was considered unnecessary to reanaesthetise the animals only to weigh them.

Analysis of the Measurements of Rate of Eruption.

A cursory examination of the individual rates of eruption, for both cut and uncut incisors, in appendix No. 3,A, shows that the figures obtained for the first two measurements, i.e. those made on day 2 and day 4, are considerably greater than the subsequent figures. The pooled means for all animals on these days are also considerably greater than all other daily pooled means.

The fact that these first two measurements were different from the subsequent figures was not surprising as BRYER (1957), who introduced this method/

method, had mentioned it. However, the fact that these initial measurements were invariably higher than the subsequent figures was surprising, as BRYER had stated that the initial measurements "fluctuated".

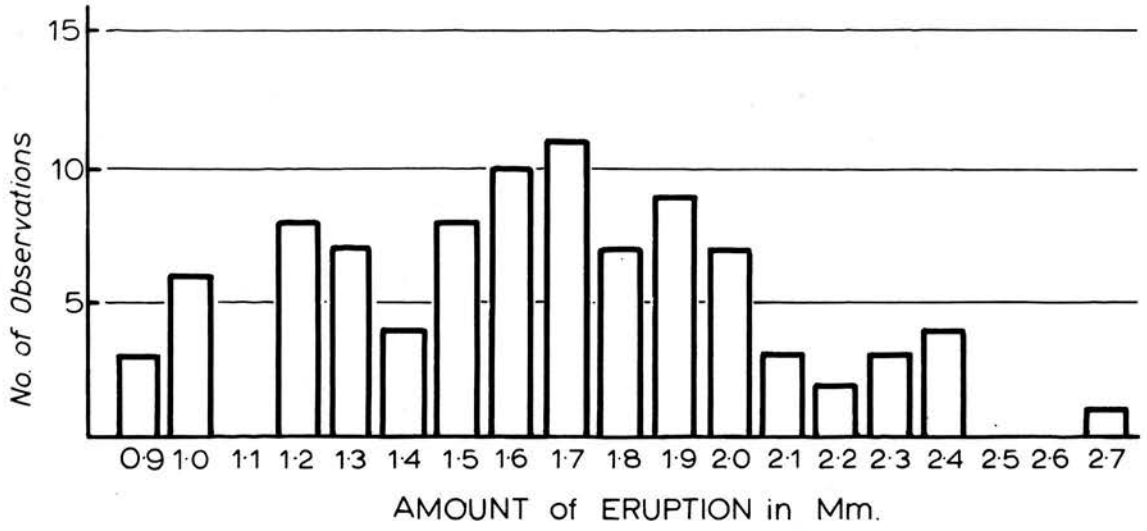
The reason for the high measurements was not apparent and it was decided to follow BRYER's practice of omitting them from the pooled figures to be used for purposes of comparisons. The explanation of the high values will be given later.

The first analysis was to ascertain whether or not the control observation conformed to a normal distribution. This was done in the same manner as described previously. Histograms of the frequency distributions were made (Figs. 45 - 48) and goodness of fit tests were applied, chi-square being calculated to assess the departure from normality. These tests are laid out in appendix No. 3, C.

It will be seen that, while the values for chi-square are not very low, they do not exceed the five-per-cent probability figures. Therefore, the usual tests for significance of differences between means of normally distributed observations are appropriate.

The means, standard deviations and numbers of observations for each group and for cut and uncut incisors are given in table Nos. 1 and 11.

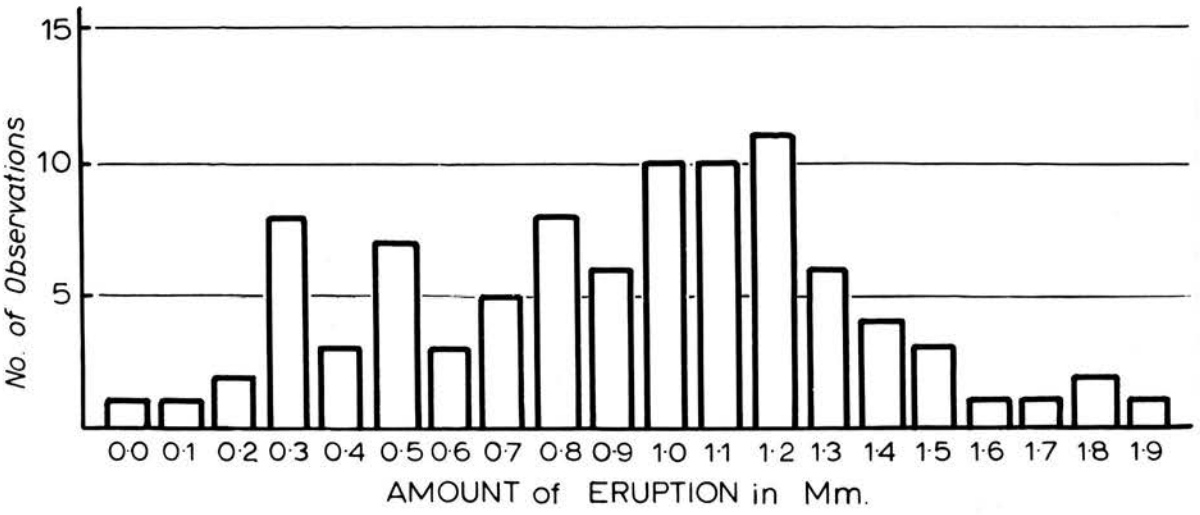
Table/



ALL GROUPS – cut incisors

Total observations = 93

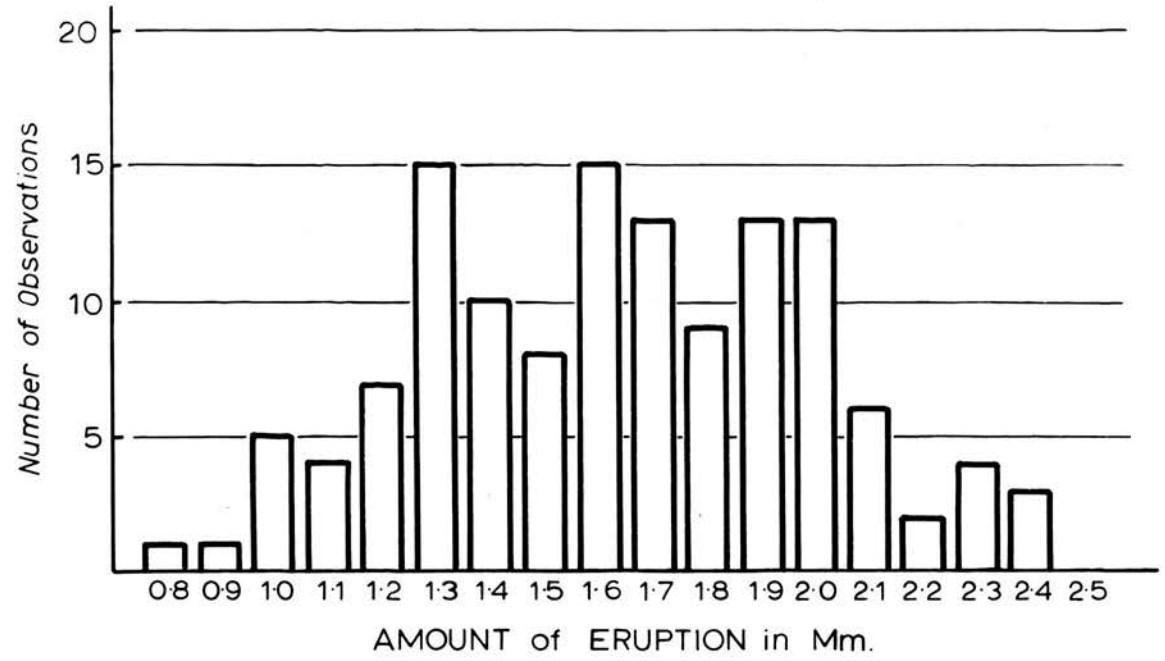
Fig. 45.



ALL GROUPS – uncut incisors

Total observations = 93

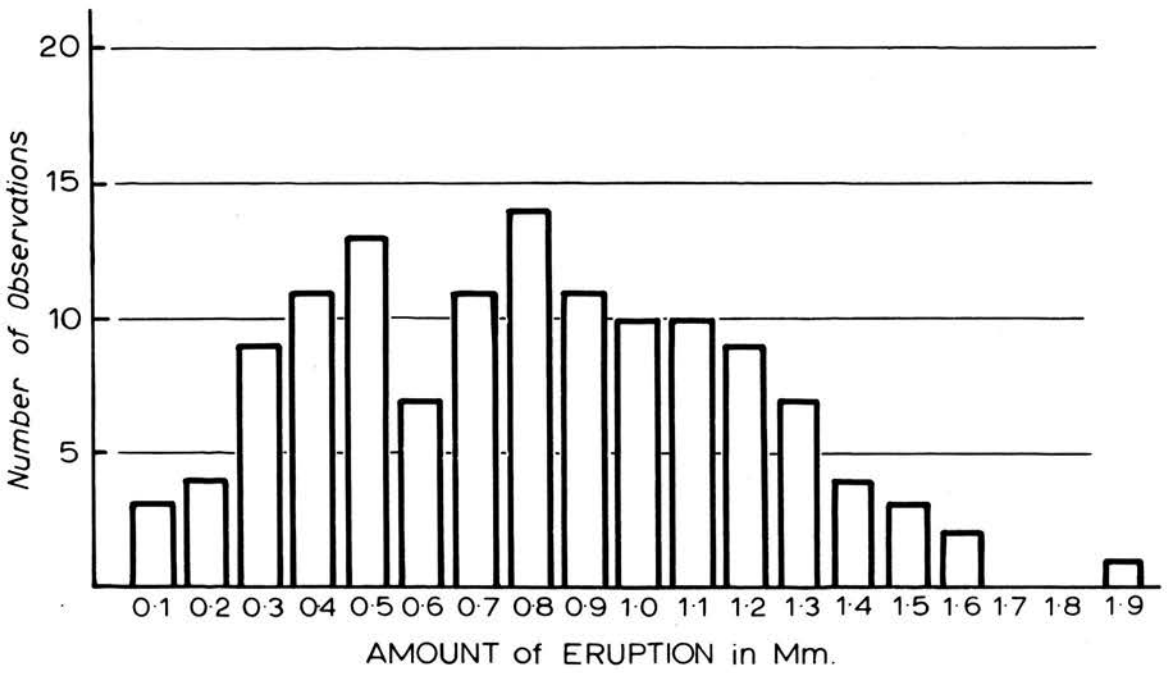
Fig. 46.



GROUP A—cut incisors

Total observations = 129

Fig. 47.



GROUP A—uncut incisors

Total observations = 129

Fig. 48.

TABLE 1.

	Day 6 - Day 10		Day 10 - Day 22		Day 22 - Day 32	
Group A (Control)	$\bar{x}$	0.86	$\bar{x}$	0.82	$\bar{x}$	0.74
	s	0.43	s	0.32	s	0.37
	n	30	n	58	n	41
Group B (Guanethidine)	$\bar{x}$	0.89	$\bar{x}$	0.94	$\bar{x}$	0.82
	s	0.41	s	0.28	s	0.39
	n	33	n	60	n	49
Group C (Hydralazine)	$\bar{x}$	0.98	$\bar{x}$	0.86	$\bar{x}$	0.85
	s	0.42	s	0.39	s	0.39
	n	30	n	59	n	45

Means ($\bar{x}$) in millimetres per 48 hours; standard deviations (s) and numbers of observations (n) for Uncut Incisors.

TABLE 11.

	Day 6 - Day 10		Day 10 - Day 22		Day 22 - Day 32	
Group A	$\bar{x}$	1.63	$\bar{x}$	1.65	$\bar{x}$	1.65
	s	0.38	s	0.35	s	0.37
	n	30	n	58	n	41
Group B	$\bar{x}$	1.62	$\bar{x}$	1.75	$\bar{x}$	1.70
	s	0.41	s	0.29	s	0.39
	n	33	n	60	n	49
Group C	$\bar{x}$	1.70	$\bar{x}$	1.67	$\bar{x}$	1.78
	s	0.41	s	0.32	s	0.35
	n	30	n	59	n	45

Means ($\bar{x}$) in millimetres per 48 hours; standard deviations (s) and numbers of observations (n) for Cut Incisors.

Tests for Significance of Differences Between Means.

t - tests were applied to assess the significance of all the differences between the means of the three groups and in each group between the means for each of the three periods, for both cut and uncut incisors.

None of these differences was found to have significance at the level of probability of p less than 0.100, with one exception. The exception was the difference between the mean rate of eruption of the uncut incisor only for period two and period three in group B, thus:-

$$S_{\bar{x}_1 - \bar{x}_2} = \sqrt{\frac{0.28^2}{60} + \frac{0.39^2}{49}}$$

$$= 0.066.$$

$$t = \frac{\bar{x}_1 - \bar{x}_2}{S_{\bar{x}_1 - \bar{x}_2}} = \frac{0.94 - 0.82}{0.66} = 1.819.$$

$$D.f. = (n_1 - 1) + (n_2 - 1) = 107.$$

From the t - tables we find that for t = 1.819, d.f. = 107, p is less than 0.100, more than 0.050.

Discussion.

It will be seen from the tests of goodness of fit to a normal distribution that the control observations do not differ significantly from normality. However the figures for chi-square are on the high side, in/

in other words the fit is not particularly good. Similarly the histograms are not very good approximations to the normal curve.

However, tests for significance of difference between means of normally distributed observations are obviously valid on statistical grounds.

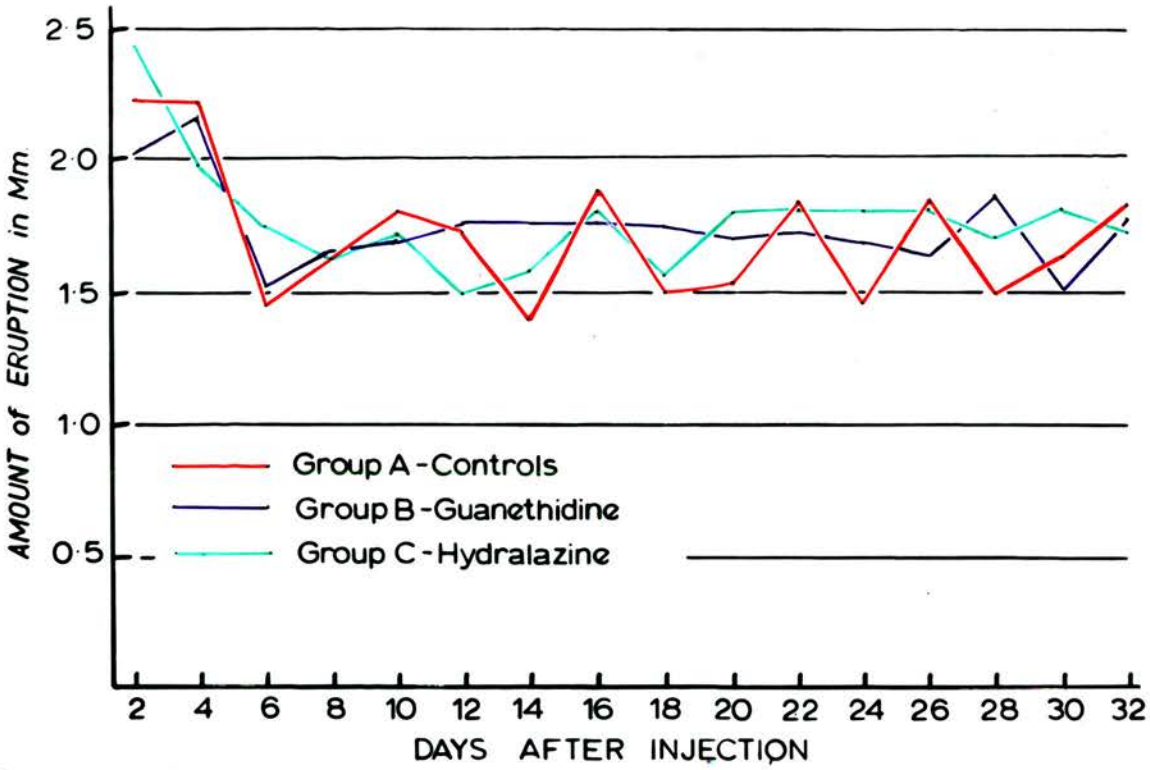
The figures obtained for rates of eruption are within the normal range for rats, and are close to those quoted by SCHUMER & WELLS (1958). They are roughly 0.2 m.m. per 48 hours lower than those found by BRYER (1957) for the cut incisors, but he used hooded Norwegian rats and this may be a species difference.

There is a large difference in the values obtained for standard deviation between this study and BRYER's. His standard deviation varied from 0.09 m.m. to 0.24 m.m. with an average of around 0.15 m.m.. The figures obtained here were between two and three times BRYER's. Again this may be a species difference. SCHUMER & WELLS do not give the standard deviation which they found.

The tests for significance of difference show that there is no significant variation between the control figures of all three groups.

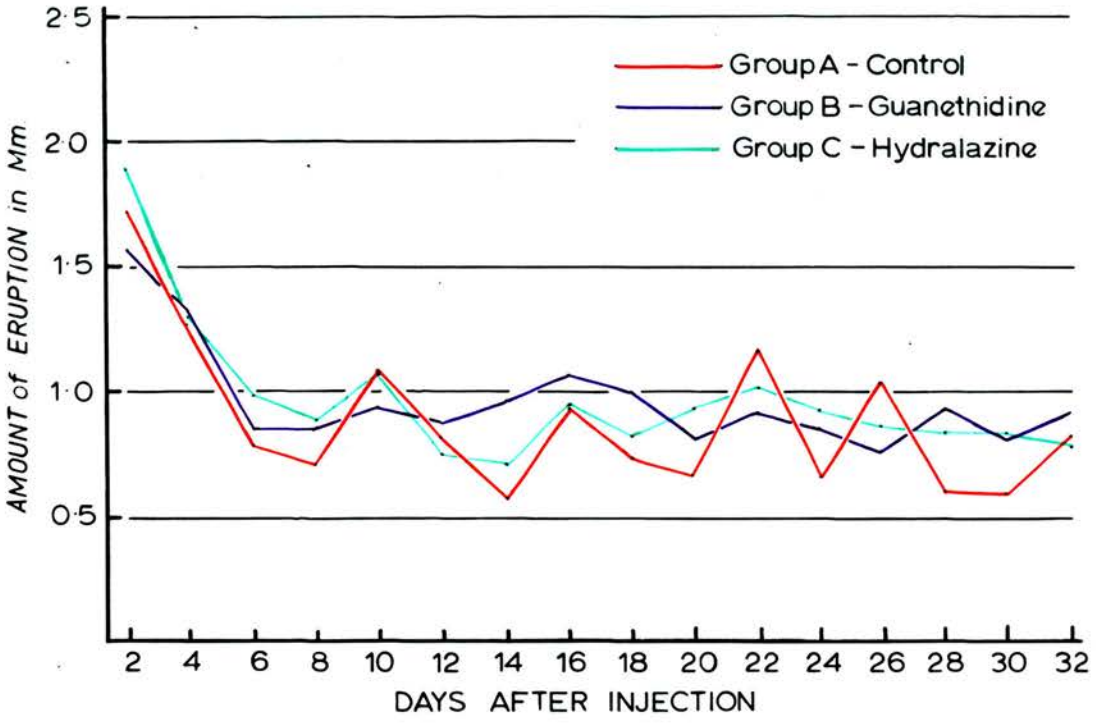
Similarly there is no significant difference in the rates of eruption of both cut and uncut incisors between the control figures, the animals receiving daily injections of normal saline, and the animals receiving daily injections of guanethidine and hydralazine.

The/



CUT INCISORS

Fig. 49.



UNCUT INCISORS

Fig. 50.

The finding of a difference of dubious significance (p less than 0.100, more than 0.050) between the pooled means of group B during and after guanethidine administration, in the total absence of any confirmatory evidence, can be ascribed to chance, or experimental error.

Fig. 49 and Fig. 50 show the graphed rates of eruption for each group.

It has been shown in experiments I and II that guanethidine and hydralazine in the dosages used caused a fall in arterial blood pressure and a rise in capillary blood pressure. If the blood pressure theory of eruption was correct, then the rate of eruption of the incisors should have increased when these drugs were administered.

During the course of this experiment, increasing doubts were felt about the reliability of the method of measurement. This was not based on the large variation which was found in the observations, as the actual individual 48 hour amounts of eruption were not calculated till the experiment was completed, so that any bias in the observer would be minimised.

The basis for the doubts rested on the following points:-

1. In practice it was found difficult to make accurate observations through the microscope of the rat incisors, as the head tended to nod in time with the respiration, the rate of which is approximately one hundred per minute in rats. This tendency varied from animal to animal but was generally more marked in the males. When the nodding was pronounced, it was necessary to have an assistant to steady the head.

2./

2. As the rat was anaesthetised, there was always some pressure on the observer to make the observations as quickly as possible, to keep the duration of anaesthesia of a minimum. There is a general agreement, gained by experience, that well administered ether anaesthesia does no harm to rats, even when repeated. However, it may have an effect on eruption rate, although this has not been measured in this work or by anyone else. It seems reasonable to assume that any effect on eruption rate would be the same for all animals used.

The chief danger with ether anaesthesia is that the irritation to the respiratory epithelium may produce excessive mucus secretion leading to obstruction, and, as has been stated above, this led to a number of deaths in this experiment.

3. Any method of measurements of eruption rate which involves marking the labial enamel leads to chipping of the incisal edge when the mark reaches this point. Normally the incisal edge is chisel shaped with a smoothly rounded labial edge which is very sharp. When the mark, no matter how shallow, reaches this edge, it fractures. The effect of this chipping in the method used is to increase the apparent rate of eruption of both incisors.

4. When one mandibular incisor has been kept cut back out of occlusion for a week or two, it tends to splay away from the other incisor. This results in inaccuracies in measurement of the distance from mark to cut surface (Y and Y1 in Fig. 4) and the more they splay, the bigger the error.

Further/

Further, when the figures were calculated at the end of the experiment, and the invariably higher values found on the first two occasions were noted, together with the large variation, doubts on the reliability of the method increased.

BRYER (1957) who introduced the method, made no mention, and showed no figures to indicate that the initial measurements were always higher than subsequently. In fact, hestated that the "initial measurements fluctuated", implying that some were below average. He also implied that this method gave values which reflected the rate of eruption alone, with the attritional factor removed. As was pointed out in the review of the literature, this is not so, although this fact was not realised when this experiment was begun.

In view of all these criticisms, it was decided that further investigation of methods of measurements of rate of eruption was indicated and that no firm conclusions could be drawn from this experiment.

Conclusion.

Although the observations gave no support to the blood pressure theory of the eruption of teeth, it was decided that no firm conclusions could be made in view of the difficulties inherent in the method of measurement of eruption rate used in this experiment.

A preliminary report of this experiment was given to the British Division of the International Association for Dental Research in April, 1961, and an abstract of the paper published afterwards (Main, J.H.P., The Relationship Between Unimpeded Eruption Rates and Blood Pressure in the Rat Incisor, J.dent.Res., 40, 1276, 1961.)

EXPERIMENT IV.

Introduction.

It was decided to attempt to develop a method of measurement of the rate of eruption of the rat incisor based on the method devised by NESS (1954, 1956) in which the crest of the alveolar bone between the incisors is used as the fixed point of reference.

The measurement of eruption rate is bedevilled by the difficulty common to all growth measurements, the lack of an absolutely fixed point. NESS argued that the crest of the alveolar bone in adult animals was sufficiently stable for relatively short periods, and that it was certainly more stable than the crest of the free gingivae which had been used very widely. It would seem to be also considerably more stable than the incisal edge of a continuously growing incisor which had been also used as the fixed point, for instance in the method used in Experiment III of this work. NESS did not adduce any positive evidence on the stability of the alveolar crest.

NESS had inserted small amalgam fillings into the mandibular incisors of rabbits and taken serial radiographs of these teeth; and he had also measured unimpeded eruption by the same method, amalgam markers being unnecessary for this.

Method/

Method Devised.

An apparatus was developed to enable serial radiographs to be taken of the rat mandibular incisors (Figs. 51 and 52).

It consisted of an acrylic holder at a distance of 24" from the X-ray source. The holder was made from a direct impression of the mandibular incisors of a rat of the age to be used, that is a three and a half month old animal. On analogy with a cephalostat, it may be called a "mandibulostat".

On the back of the mandibulostat a slot held a small, specially made casette in which lay the radiographic film which was specially cut to fit. The casette was held in position by a metal plate attached to a rigid brass bar which could rotate to enable the optimum angulation of the cassette to be determined. At the beginning of each series of radiographs it was locked in one position.

A small block of acrylic was cemented over the position occupied by the incisal edges, to reduce the amount of radiation reaching them, as otherwise they were over exposed.

Techniques:-

1. The rat was anaesthetised by open ether. Single dose anaesthesia was adequate as the operation was very brief.
2. The mandibular incisors were inserted into the mandibulostat, being pushed up as far as they would fit comfortably. At the same time the casette was placed in the animal's mouth, care being taken not to trap the tongue beneath it.

3./

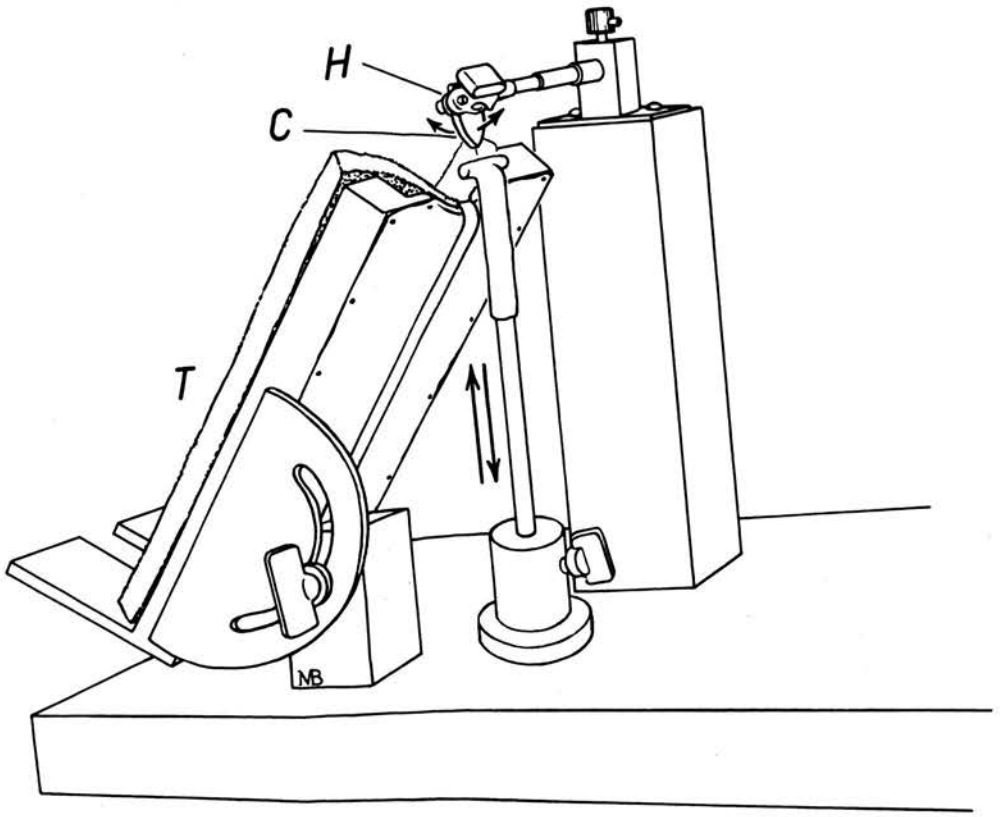

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Fig. 51.

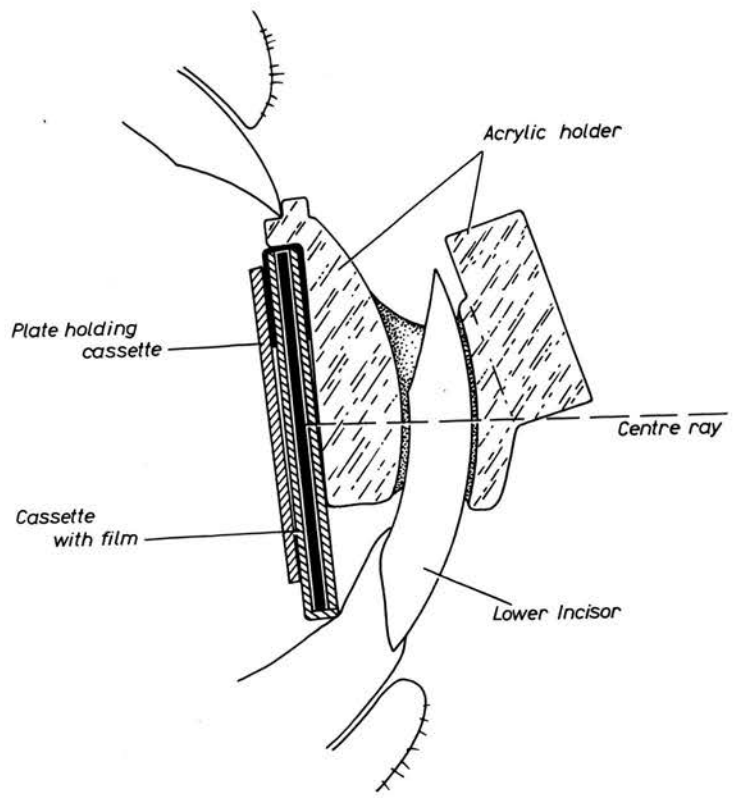
Acrylic holder
Plate holding cassette
Centre ray
Cassette with film
Lower Incisor

Fig. 52.

3. The film was exposed. The exposure time used was 0.6 secs. at 65 kV and 15 m.a., at a tube/target distance of 24".
4. The rat was removed from the mandibulostat, the left incisor cut back out of occlusion and the radiograph repeated, the whole process taking about 2 minutes.
5. 48 hours later the procedure was repeated.

The films were then developed, mounted on slides, and measurements made by projecting them on to a grid at a magnification of times forty. The measurements were made to a final accuracy of 0.05 m.m..

Typical films made by this method are shown in Figs. 53 and 54. It will be seen that as well as measuring the unimpeded rate of eruption of the left incisor, this method also enables measurements of the uncut incisor to be made and the stability of its length assessed.

A number of experiments were then carried out to assess the reliability of this method.

Fig. 53.

Radiograph taken by the method described in the text. X 14.

Fig. 54.

Radiograph of same animal taken 48 hours after Fig. 53.

EXPERIMENT IV A.

Object: To investigate histologically the effect on the crest of the interincisal alveolar bone of the rat of cutting one incisor out of occlusion.

Materials and Methods.

Ten male rats of the same strain as those used in the previous experiments were used. They were all three and a half months old and were treated in the following manner:-

No. 1 - control - no operative treatment.
" 2 - control - no operative treatment.
" 3 - left mandibular incisor cut back out of occlusion every second day for 14 days.
" 4 - as for No. 3.
" 5 - left mandibular incisor cut back out of occlusion every second day for 28 days.
" 6 - as for No. 5.
" 7 - as for No. 5; in addition from day 10 to day 20 had guanethidine, 10 mgm./Kg./day, intra-muscularly.
" 8 - as for No. 7, but had hydralazine, 8 mgm./Kg./day from day 10 to day 20.
" 9 - as for No. 7, but had demecolcine 1 mgm./Kg./day intraperitoneally.
" 10 - as for No. 7, but had triethylene melamine, 0.2 mgm./Kg./day, intraperitoneally.

The/

Fig. 55.
Coronal section through interincisal bone and mandibular incisors, control rat number 1 (both incisors in occlusion); H.& E., X 32.

Fig. 56.
Coronal section through interincisal bone and mandibular incisors, control rat number 2 (both incisors in occlusion); H.& E., X 32.

The animals were killed on the days stated, the mandibles dissected free, decalcified in formic acid, and then processed in the usual manner for histological investigation. Serial sections were cut coronally through the mandibular incisors to show the teeth and the tissues between them and every tenth section stained haematoxylin and eosin.

Results.

The thickness of the alveolar bone varied with the depth of the section and the two alveoli were parts of different bones as the mandible is in two parts in the rat, united in the mid line by fibrous tissue.

From the sections of the control, unoperated, animals it could be seen that the crest of the interincisal alveolar bone showed a pattern of incremental lines on the tooth surface with the lines running at an angle to the long axis of the bone, so that the most recent increments were right at the top, on the tooth side. The incremental lines were often difficult to see but in a few sections they were very obvious (Fig. 55).

Simultaneously modelling resorption was occurring on the mesial surface of the bone, and this was nearly always easily seen, by virtue of the presence of Howship's lacunae or osteoclasts (Figs. 56 and 57). No evidence of resorption was found near the crest of the bone.

The/

Fig. 57.
Mandibular incisor, periodontal membrane and alveolar bone; rat number 2 (control); H. & E., X 80.

Fig. 58.
Coronal section through interincisal bone and mandibular incisors; rat number 4. (Incisor shown on right had been cut out of occlusion for 14 days).

The effect of cutting one incisor out of occlusion was to increase the amount of resorption taking place on the mesial side of the alveolar bone of that tooth. This is shown in Fig. 58. In very few sections was any difference from the controls found in relation to the alveolar bone of the uncut incisor.

It seemed that in addition to the increased resorption, there was also increased deposition of bone at the crest, but this was not so clearly seen, as incremental lines were not so obvious as in the control animals. It was not possible to measure the height of the bone on the sections for the purpose of comparison, as the angle at which sectioning had been carried out varied.

No differences whatever were seen between the section of the animals which received the drugs and the animals which had no drugs. There were no detectable differences between the sections of the animals which had had the incisor cut back for 14 days and those cut back for 28 days (Figs. 59 - 61).

Discussion.

All animals which have continuously erupting incisors continue to grow generally through their lifespan, and the pattern of bone deposition and resorption seen in this study in the control animals is consistent with this fact. That is to say the mandible of the rat grows in length by deposition at the free edge of the alveolar process surrounding the incisors.

That/

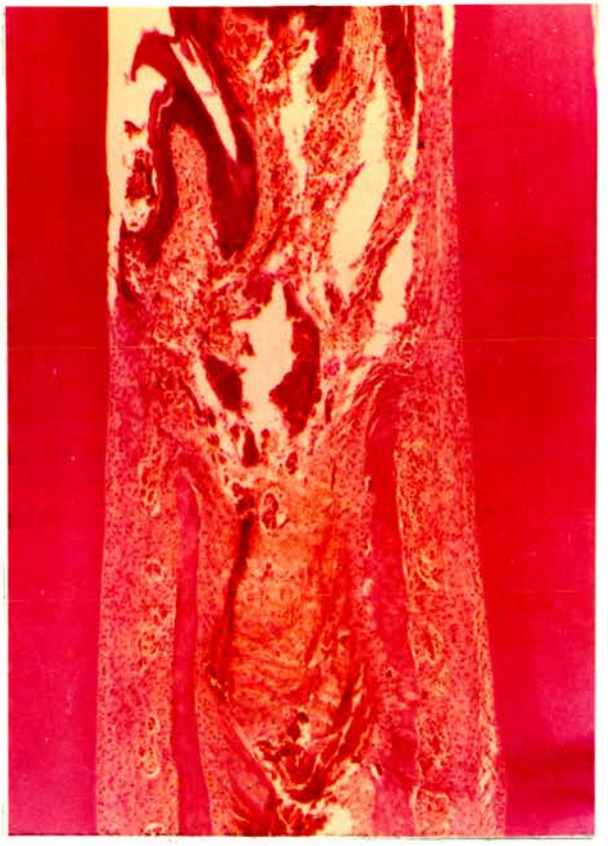

Fig. 59.
Coronal section through interincisal bone and mandibular incisors; rat number 7. (Incisor shown on right had been cut out of occlusion for 28 days).

Fig. 60.
Coronal section through interincisal bone and mandibular incisors; rat number 8. (Incisor shown on right had been cut out of occlusion for 28 days).

That such growth is slow is shown by the closeness of the resting lines (WEINMANN & SICHER, 1955).

The effect of cutting one incisor out of occlusion, as well as causing an increase in the rate of eruption of that tooth, is to cause an increase in the rate of bone resorption certainly and of bone deposition almost certainly in its associated alveolus.

Whether or not this increase would affect the height of the alveolar crest as seen on radiographs in the eruption rate measurements, it is not possible to be certain by this method of investigation. However, as it could affect the measurements, while it was known that the alveolar bone around the uncut tooth was not affected by the operative procedure, it was concluded that measurements should be made from the crest of the bone of the uncut incisor.

The growth which occurs in relation to this crest would no doubt slightly reduce the amount of eruption calculated for each two day period, but this error was so small as to be insignificant.

These four drugs were given to make certain that none of them produced any specific effect on the bone, as they were to be used in subsequent experiments.

Conclusions.

When one mandibular incisor of the rat is cut back out of occlusion, the turnover rate of the alveolar bone of that tooth is increased. This may result in the alveolar crest growing higher than that of the uncut tooth/

Fig. 61.

Coronal section through interincisal bone and mandibular incisors; rat number 10. (Incisor shown on right had been cut out of occlusion for 28 days).

tooth, but it was not possible to decide this with certainty.

No different or additional effects were produced by the administration of the drugs listed previously.

The practical conclusion is that measurements in the radiographic method of measuring rat incisor eruption rate should be made from the crest of the alveolar bone, of the uncut incisor, as this provided a point as nearly "fixed" as is possible in growth measurements.

EXPERIMENT IV B.

Object: To ascertain whether elevating or depressing forces applied to the crown of the rat mandibular incisor would affect its apparent "length" as found in the radiographic method of measurement of eruption rate.

Introduction.

It is well known that teeth are able to move laterally and vertically in their sockets under conditions of physiological function. Until recently, the amount of movement possible had not been measured. PICTON (1962) devised a method of measuring this movement using sensitive apparatus which employed strain gauges to assess the movement. He has shown that in human teeth under vertical forces of 2 Kg., movements of up to 69 microns can occur.

In the radiographic method of measurement, the mandibular incisors of the rat are pushed into the mandibulostat, and in many cases considerable pressure is applied between the rod supporting the chin and the incisors which are held in the tapering holes of the acrylic block. It was felt that the possibility of this producing a depression or, probably less commonly, an elevation of the incisor in its socket must be investigated. This experiment was carried out before PICTON's work was published, but in view of the differences between human teeth and rat mandibular incisors, his results could not be compared directly in any case.

Obviously/

Obviously, any movement of this type, if sufficiently great, occurring in the periodontal membrane, would affect the accuracy of the measurements.

Materials and Methods.

The apparatus shown in Fig. 62 was constructed. To some extent, it is a modification of the apparatus described in the introduction to Experiment IV. It differed however, in that the rat, anaesthetised with barbiturates, was placed in position and the mandible held firmly by the spring loaded clamp which bore under the mandible and on the molar teeth, leaving the incisors untouched. The design of the clamp is shown more clearly in the inset drawing.

The casette holder, as it was not now composite with the acrylic block for repositioning the incisors, was very much smaller and casettes could be changed without removing the animal from the apparatus.

The position in which the rat is held is not reproducible, but all measurements were made without removing the animal from the apparatus.

Forces were applied to the right mandibular incisor through the part of the apparatus shown on the left of the drawing. The incisor was held firmly by a narrow band of thin stainless steel which was tightened by means of the screw, A. Vertical forces could then be applied to the tooth by hanging weights directly on to the bar (B) which tended to depress the tooth in its socket, or by attaching weights to the bar B via the pulley system which tended to elevate the tooth.

The/

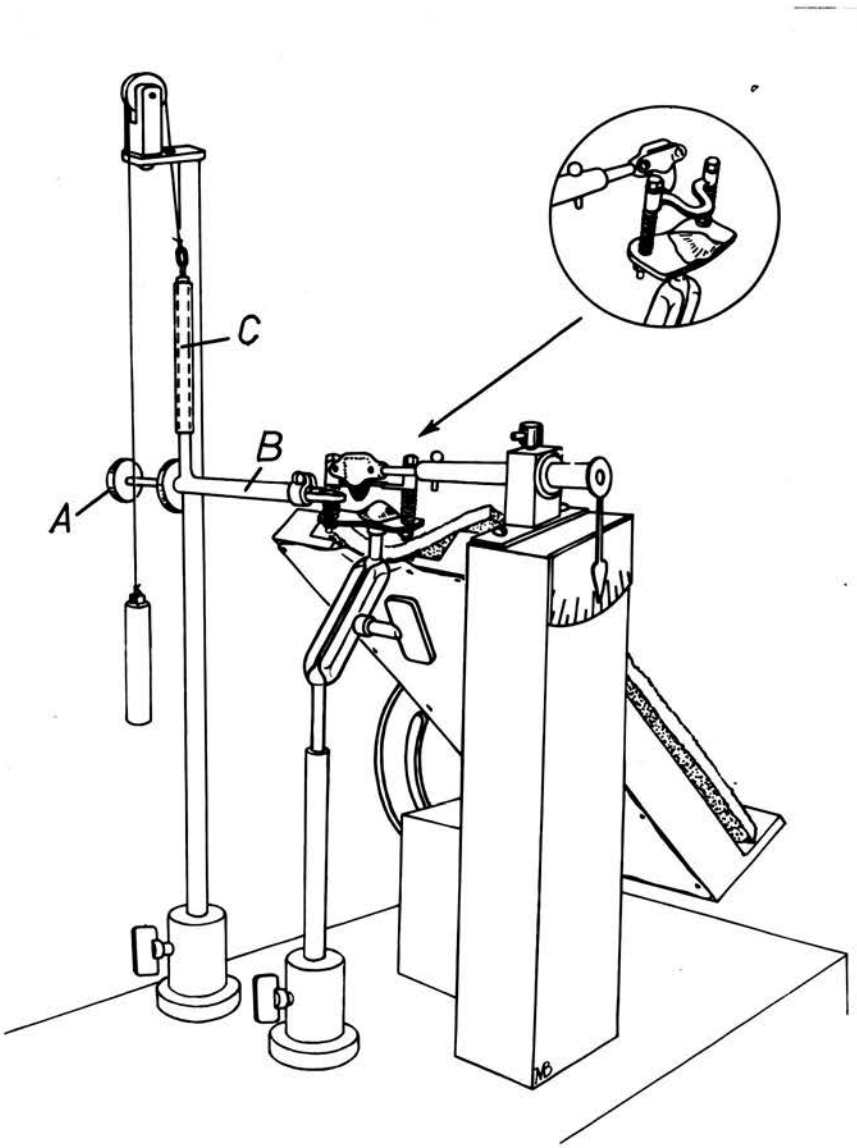

Fig. 62.

The forces could only act vertically as this was the only line of movement possible for the bar (B) by virtue of the piston and cylinder (C) arrangement of its support.

Films were made of six rats, 2 male and 4 female, under conditions of 1. no loading, 2. a 300 gm. elevating force, 3. a 300 gm. depressing force, and 4. no loading.

300 gms. was chosen as it seemed unlikely that any greater force than this was used when inserting the rat incisors into the mandibulostat.

Results.

TABLE 111.

Rat	No Load	300 gm. Depressing Load.	300 gm. Elevating Load.	No Load
Male No. 1	7.7	7.7	7.7	7.7
" " 2	7.0	7.0	7.0	7.0
Female No. 1	6.5	6.5	6.5	6.5
" " 2	7.3	7.3	7.3	7.3
" " 3	6.7	6.7	6.7	6.7
" " 4	6.5	6.5	6.5	6.5

Distance from crest of interincisal alveolar bone crest to tip of incisal edge of left mandibular incisor in centimetres.

(Uncorrected for radiographic distortion).

Discussion/

Discussion.

It is obvious that forces of the magnitude used do not produce any detectable movement of the tooth in its socket. It seems likely that the reason for this finding is that the movements which occur are simply too small to be detected by the method used, and may well be of the order of those found by PICTON in human teeth under comparable loadings, i.e. from 10 microns to a maximum of 70 microns.

Conclusion.

Elevating or depressing forces applied to the crown of the rat mandibular incisor when it is inserted into the mandibulostat in this method of measurement of eruption rate do not cause movements sufficiently great to affect the accuracy of the method.

EXPERIMENT IV C.

Object: To measure the errors inherent in the radiographic method of measurement of eruption rate; to find out the mean and standard deviation of eruption rate using this method: and to measure the stability of the uncut incisor.

Methods:

The method used was as described in the introduction to this Experiment. The measurements were made from the incisal crest of the alveolar bone around the uncut incisor.

Materials:

Radiographs were taken of the mandibular incisors of a group of twelve rats, eight females and four males, of the same strain as those used in the previous experiments. They were siblings and all three and a half months old at the beginning of the experiment.

Measurements were made every second day for twenty two days.

Results.

The animals all remained healthy throughout the course of the experiment.

On the first day of measurement, one animal, B.3, was accidentally omitted. A few other measurements were lost due to accidents in the processing of the film.

Radiographic/

Radiographic Distortion.

As has been explained previously, the eruption of a rodent incisor occurs along a spiral course while the measurements of this curve are expressed as if it were linear, and this method is no exception.

Furthermore, the curved form of the rat incisor, when projected on to a film, results in slightly different amounts of elongation or shortening at different points on the film. The actual amount of this distortion was measured by placing small pieces of metal strip, of known length, into the acrylic holder at different levels and making films on the apparatus. This gave an accurate measure of the distortion produced at a number of points on the curvature of the holder.

In practice it was found that with the degree of angulation of the casette used in this experiment, the incisor was elongated by 12% at the very bottom of the film while at the top the elongation was 4%. This is perhaps better explained by reference to Fig. 63.

As the cut incisor was cut back to slightly different levels on each animal, the amount of elongation varied. A grid was drawn on paper showing the amount of elongation at each level on the film and this was used to measure the average elongation for each cut incisor over each two films.

In practice, it was found that this elongation varied between 6 and 9 per cent.

The/

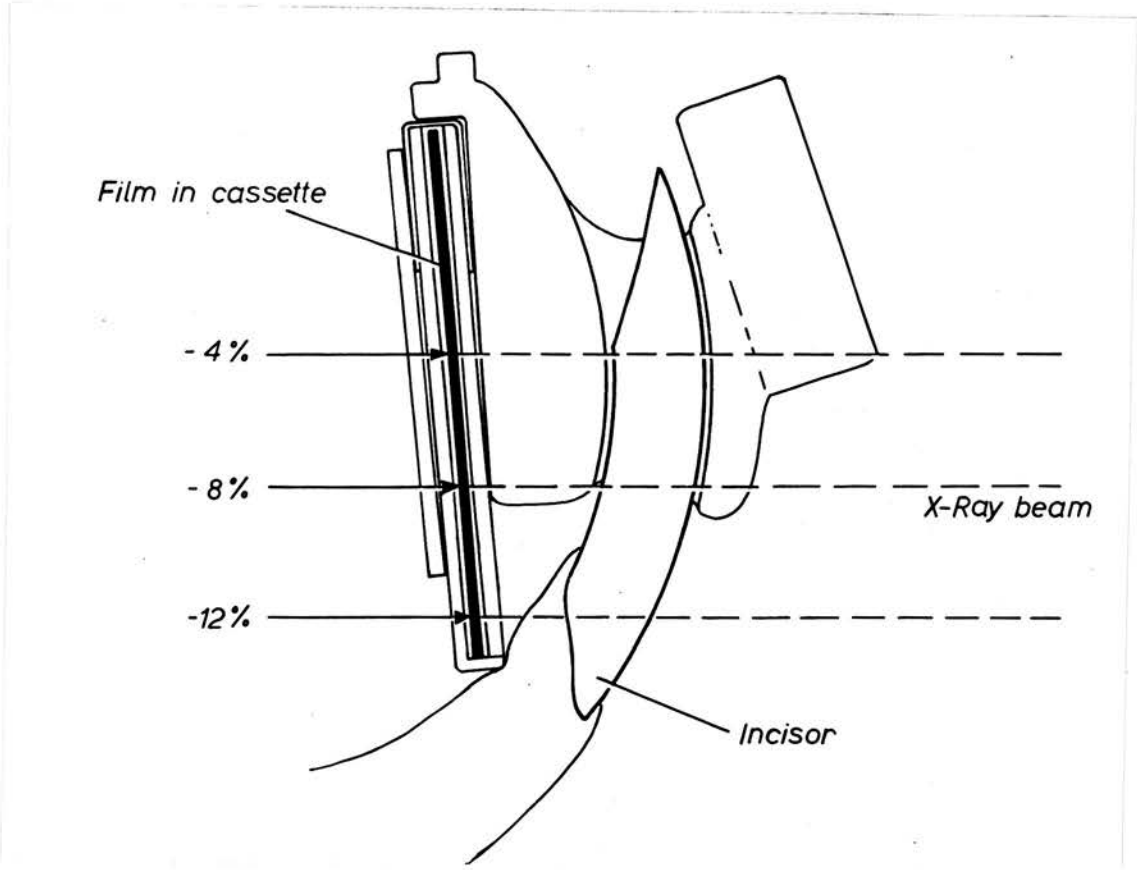

Fig. 63.

The results were then corrected for this elongation and the final figures are shown in appendix No. 4, A.

The apparent length of the uncut incisors are given in appendix No. 4, B (uncorrected for radiographic distortion).

Analysis of the Results.

1. Accuracy of Method.

The measurements were made on the grid to an accuracy of plus or minus 0.05 m.m.. Comparison of the two measurements of the lengths of the uncut incisor made each day, gives an assessment of the inaccuracies of the method.

The differences in the apparent lengths of the uncut incisors are in appendix No. 4, B. Analysis of these differences shows that the mean difference is plus 0.0164 m.m. with a standard deviation of plus or minus 0.0749 m.m..

If the average length of uncut incisor is taken as 6.5 Cms., then the percentage error had a mean of plus 0.25% and a standard deviation of plus or minus 1.15%. On the same assumption, the measurements were made on the grid to an accuracy of 0.77%.

When these possible errors are added we have a range of mean error from minus 0.77% to plus 1% with a standard deviation of 1.15%.

2./

2. The variation in length of the uncut incisor.

An examination of the measurements of the uncut incisor shows that the initial length was greater than the other lengths in all cases but one, animal B, 2. In general the subsequent measurements were reasonably close to one another but quite different from the first measurements.

In view of this, the mean length of each incisor was calculated for the period of the experiment, but excluding the first measurement. The percentage of this mean length was then calculated for each animal for each day. Where the two measurements for one day differed, the average of the two was used to calculate the percentage of the overall mean.

These percentage lengths are given in appendix No. 4,B.

Omitting the measurement made on the first day, these percentage lengths vary with a standard deviation of 2.89%.

The mean decrease in length from the length originally measured on the first day to the mean length for all subsequent measurements was 19.4%.

3. The rate of eruption of the cut incisor.

The observations of rate of eruption were treated in the same manner as those found in Experiment III when the previous method of measurement had been used, that is, a histogram of the frequency distribution was made (Fig. 64) and a goodness of fit test to a normal distribution carried out (appendix No. 4,A).

Chi-/

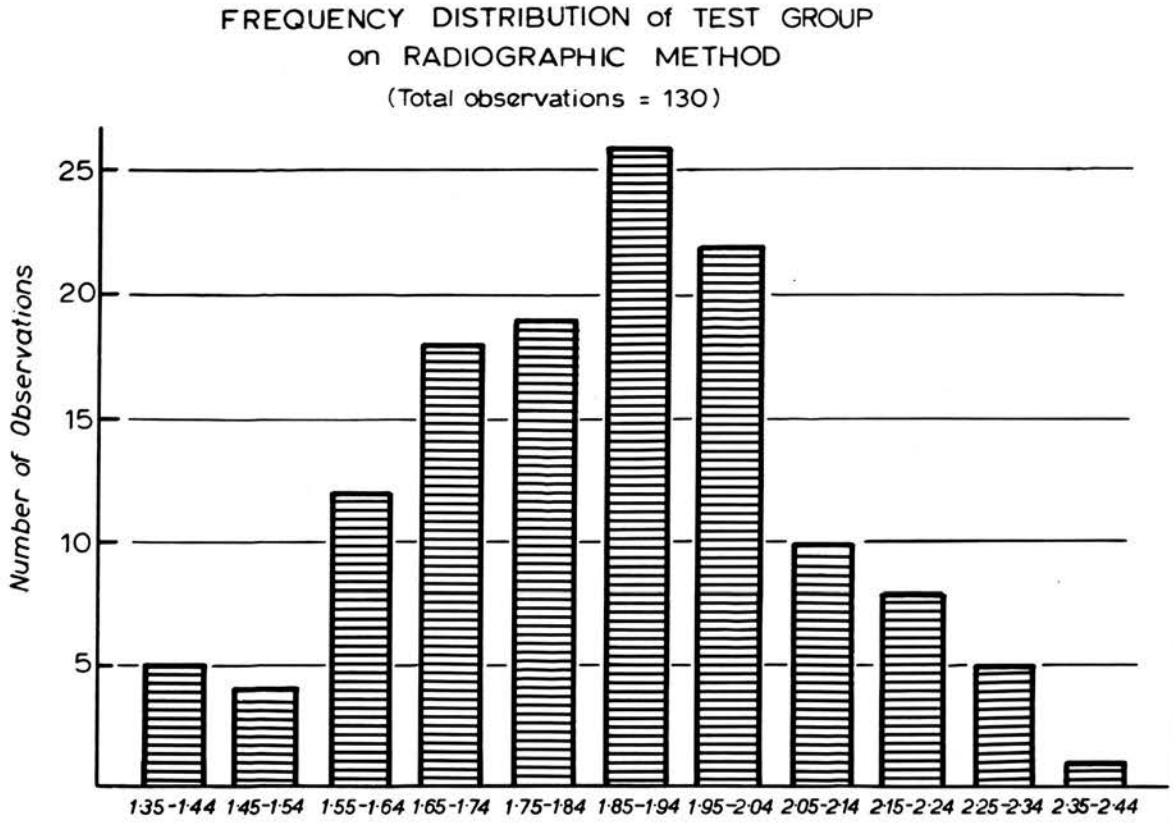
FREQUENCY DISTRIBUTION of TEST GROUP
on RADIOGRAPHIC METHOD
(Total observations = 130)
Number of Observations
25
20
15
10
5
1·35-1·44
1·45-1·54
1·55-1·64
1·65-1·74
1·75-1·84
1·85-1·94
1·95-2·04
2·05-2·14
2·15-2·24
2·25-2·34
2·35-2·44

Fig. 64.

Chi-square was calculated to be 3.29.

The pooled mean rate of eruption was 1.85 m.m. per 48 hours with a standard deviation of 0.22 m.m..

An analysis of variance was then done (Table No. 3) and appendix No. 4,C.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio
Animals	0.8465	11	0.0770	2.31
Days	1.5786	10	0.1579	4.73
Residual	3.6753	110	0.0334	
Total:	6.1004	131		

Table III - Analysis of Variance.

From the variance ratio table, the 5% point with $f_1 = 110$, $f_2 = 11$ is 2.45; and with $f_1 = 110$, $f_2 = 10$ it is 2.59. The 1% point with $f_1 = 110$, $f_2 = 10$ is 4.01.

It should be noted that two average values have been substituted in the variance analysis in place of the two observations which were lost due to faults in technique.

Discussion.

1. Accuracy of Method.

As was shown in the analysis of results, the total standard deviation resulting from errors of method was 0.075 m.m. when this error was assessed by comparing the apparent lengths of the uncut incisor on each/

each measuring day. These lengths should, of course, have been identical.

The differences in apparent lengths arose from various sources. It may have been that the incisors were not repositioned in exactly the same position in the mandibulostat, and this doubtless occurred in many cases to varying degrees. In other instances, the reference point on the alveolar bone was not absolutely clearly defined, and this would lead to further inaccuracy. Similarly the tip of the incisal edge was not invariably clear, and this was also true of the cut edge when measuring to it. In other cases, slight variations in radiographic density occurred due to variations in the film, exposure time and development process.

The standard deviation of the total error due to the method expressed as a percentage of the average length being measured, 6.5 cms. was 1.15%, and therefore the range was plus or minus 3.45%.

It should be borne in mind that these figures assume the stability of the crest of the alveolar bone.

It is difficult to compare this figure with those quoted in the literature for similar errors of method. TAYLOR & BUTCHER (1951) stated that they reduced their error of measurement to plus or minus 0.02 m.m., however they went on to say that this did not take into account variations in the position of the gingival crest, and these possible variations constitute the greatest objection to their method. NESS (1954) gave a standard deviation of 0.043 m.m. for errors of method for any one measurement/

measurement. In a later paper (NESS, 1956), he described how this figure was derived. Four films were taken at one time on each of four rabbits and the variations between these sixteen films analysed. This is a somewhat less exacting test of accuracy than the one used in this work, and this probably explains the smaller error found.

It is impossible to measure the error of method using BRYER's technique.

2. Variation in length of Uncut Incisor.

The marked reduction in length of the uncut incisor which was found 2 days after cutting the other out of occlusion is presumably the result of increased attrition on that tooth. This observation was also made by NESS (1956) in the rabbit, and he found a similar degree of reduction in length. NESS found that it occurred invariably. The fact that it was not found in one animal (B.2) in this series is most probably because accidental fracture of both incisors had happened in this animal a few days previously.

However, TAYLOR & BUTCHER (1951) did not find this reduction in length of the uncut incisor in the rat, when the other incisor was cut out of occlusion.

It will be noted that the length of the uncut incisor, after its initial shortening, varied from day to day, the variation having a standard deviation of 2.9%. It is therefore obvious that when the length of the uncut incisor is assumed to be stable, as in BRYER's method, the measurements made have an inherent error of method with a standard deviation/

deviation of 2.9%, and a range of plus or minus 8.7%.

3. The rate of eruption of the Cut Incisor.

An examination of the frequency distribution histogram (Fig.64) shows that the curve is a much closer approximation to a normal distribution than any of the frequency distribution histograms obtained when BRYER's method of measurement was used (Figs. 45 - 48).

It will also be seen that while the mean eruption rate is similar to that obtained by BRYER's method, the standard deviation is much smaller, approximately half.

The closer approximation to a normal distribution is clearly shown by the goodness of fit test which gives a chi-square of 3.29, which compares favourably with the chi-squares previously found of 5.61, 10.11, 8.19 and 8.17.

These three facts, the closer approximation to a normal distribution of the frequency distribution, the much lower value of chi-square in the goodness of fit test, and the lower standard deviation, strongly support the proposition that this method of measurement of eruption rate is much more accurate than the method used in Experiment III. The rate of eruption of a continuously growing incisor is a phenomenon which can be broadly classified as an example of biological growth, and all biological growth rates are expected, on a priori reasoning, to be normally distributed. The observations made in this experiment are so close to being normally distributed that, taking into account experimental error and/

and the effects of sampling, it seems that there is no room for doubt that eruption rate is a biological function which is normally distributed.

The reduction in standard deviation or range in the radiographic method observations, as compared to the direct observation method, is further evidence of the inaccuracy of the latter. It has the further effect of reducing the standard error of the mean and therefore reducing the number of observations necessary for significant differences between means.

ANALYSIS OF VARIANCE.

The variance analysis shows that there is no significant variation between animals but that there is a significant variation between the days.

A variance analysis of this type has been carried out by only one other worker - NESS (1956) - and his experiments were carried out on rabbits which were not siblings and not all of the same age. He found, not surprisingly, that the variation between animals was significant.

The fact that the rats used in this work were siblings of exactly the same age doubtless explains the absence of animal variations.

Examination of the variance analysis table in appendix No. 4,C, shows that the significant variation between days was derived mainly from the high values found on days 2, 4, and 8, and the low value on day 16. A somewhat/

somewhat similar result was found by NESS in the rabbit. After cutting one incisor out of occlusion, he found the well known increased eruption rate of that tooth. He noticed, however, that the graph of these high values was not flat topped but showed an initial rise to a peak over the first 7 days, subsequently levelling off to a slightly lower rate.

It would appear that a similar phenomenon occurs in rats, that is the rate of eruption of an incisor cut out of occlusion reaches a peak value during the first week and then the rate falls slightly.

The standard deviation calculated from the residual variance is 0.15 m.m. which is approximately twice the experimental error included in it.

The observations made in this experiment explain the finding of high values for amount of eruption on the first two measurements using BRYER's method. The very high first measurement, invariably the highest of all, was the result of the reduction in length of the uncut incisor being added to the amount of eruption of the cut incisor, which was itself higher than the mean value. The measurements made on the fourth day were higher because the cut incisor was still erupting at its maximum or peak rate, and it may be that there was also some effect from increased attrition rate.

Conclusion/

Conclusion.

The method of measurement of the rate of eruption of the rat mandibular incisor devised was more accurate than any other previously described method, and the inaccuracies due to the method have been measured. It successfully removed the attritional factor from eruption measurements.

The measurements of the radiographs should always be made from the crest of the alveolar bone of the uncut incisor.

EXPERIMENT V.

Introduction.

Having developed a more accurate method of measuring eruption rate, and in view of the known inaccuracies of the method previously used, the experiment to measure the effect of hypotension on eruption rate was to be repeated.

As it had been shown that a smaller difference in the mean eruption rate could be statistically significant, it was possible to use smaller numbers of animals than in the previous experiments.

This, in turn, made it possible to carry out another experiment simultaneously with the hypotension experiment and it was decided to investigate the effects of nucleotoxic drugs on eruption rate, as it was hoped that this would provide information relevant to the cellular proliferation theory of tooth eruption as put forward by SICHER (1942a, b) and O'BRIEN et alia (1958) among others.

After some preliminary investigations, colchicine and triethylene melamine were selected as the most suitable anti-mitotic drugs for this purpose, and the first part of the experiment was designed to investigate the effects of these drugs on the tissues concerned.

Object.

To investigate the effects on the dental tissues of demecolcine and triethylene melamine.

Materials/

Materials and Method.

1. Drugs.

There is no need to consider here the vast amount of research which has been carried out in recent years into nucleotoxic drugs. Triethylene melamine and colchicine were chosen from the large numbers of such drugs available because (a) they were from different categories of anti-mitotic drugs, (b) in comparison to many others of this type, a reasonable amount of information was available on their action. Triethylene melamine is a member of the group which DUSTIN (1947) categorised as a radio-mimetic substance, colchicine is the leading member of the other group which DUSTIN described as acting primarily on the spindle of the dividing cell.

COLCHICINE.*

This is a drug which has been known pharmacologically since early times. Its curious action in gout will not be discussed here, but only its effect on cell division. It is derived from the meadow saffron (colchicum autumnale).

The effects of colchine on cell division was first described by DUSTIN in 1925, and the effect produced, namely the arrest of cell division in metaphase is often called the "DUSTIN effect". The action is thought to be exerted essentially on the achromatic substance of the cell, notably the spindle fibres necessary for normal chromosome division, which fail to develope. INOUE (1952) showed that in sea-urchin eggs the action of colchicine was/

* The demecolcine used in this and subsequent experiments was obtained through the generosity of A.B. Tattersall Esq., of Ciba Laboratories Ltd., Horsham, Sussex.

was to disorganise the orientation of the micelles in the astral rays and spindle fibres, probably by breaking down some chemical bond between the spindle micelles. The chromosomes themselves usually remain intact after application of low concentrations of the drug.

SANTAVY & REICHSTEIN (1950), working at the Ciba Laboratories in Basle, isolated a number of alkaloids from the plant colchicum autumnale which are similar to colchicine in structure. One of these is demecolcine and this drug was used in the following experiments as it has a higher therapeutic index than colchicine itself.

Demecolcine is desacetylmethylcolchicine. It is a yellow, crystalline substance, soluble in water to 2%. Like colchicine, it is found in the tubers, leaves and seeds of the meadow saffron.

Toxicity of Demecolcine.

The pharmacological properties of this drug were studied by SCHAR et alia (1954). They found that demecolcine was roughly 30 times less toxic than colchicine itself and that rats could tolerate 4 mgm./kg. demecolcine daily for 3 weeks. Like colchicine, demecolcine is less toxic when given parenterally than when given orally.

Effects of Tissues.

SCHAR et alia (1954) found that demecolcine has the same anti-mitotic effect as colchicine. They found that it decreases the total white cell count in experimental animals. In the bone marrow, cell disintegration and nuclear pyknoses are found in the rat or rabbit after 1 mgm./kg. per day for 7 days. Similarly spermatogenesis is severely affected and the intestinal mucosa shows large numbers of arrested mitoses after only 24 hours.

In/

In general, colchicine and demecolcine affect most those tissues which have a high rate of cell turnover.

Mode of Action.

Little is known of the effect of colchine at the molecular level. KOHLI et alia (1952) found that doses of the drug, many times larger than those necessary to inhibit mitosis, had no effect on the oxygen uptake or carbon dioxide production of cells in culture, and concluded that colchine exerts its action through an enzyme system other than the respiratory.

BLOCH (1953) has shown by work again on tissue culture that colchicine does not directly inhibit the synthesis of desoxyribonucleic acid.

Excretion.

The excretion of radio-active colchicine in mice was studied by WALASZEK & BACK (1952) and appreciable amounts were found in both faeces and urine. The bulk of the drug was excreted in the first 24 hours but quantities were still present up to the fourth 24 hour period.

Clinical Use.

Demecolcine has been used in leukaemias, Hodgkin's disease, lymphosarcoma etc. but produces only transient relief of symptoms.

Triethylene Melamine (T.E.M.) *

This drug is the most active derivative of ethyleneimine and its pharmacological actions were described by HENDRY et alia (1951).

* Triethylene Melamine used in this and subsequent experiments was obtained through the generosity of Dr. A.L. Walpole, Pharmaceutical Division, Imperial Chemical Industries Ltd., Alderley Park, Macclesfield, Cheshire.

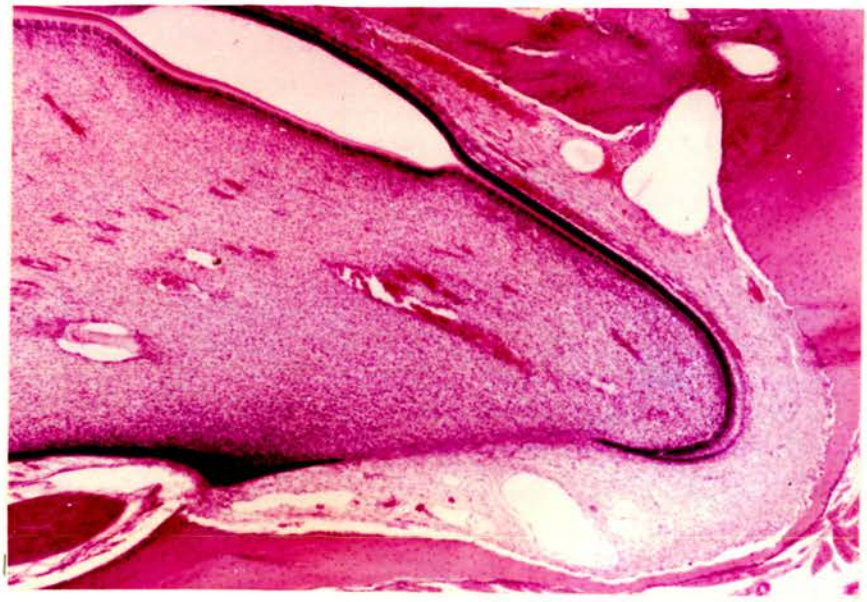

Fig. 65.

L.S. through base of pulp of mandibular left incisor (cut out of occlusion), control; H. & E., X 20.

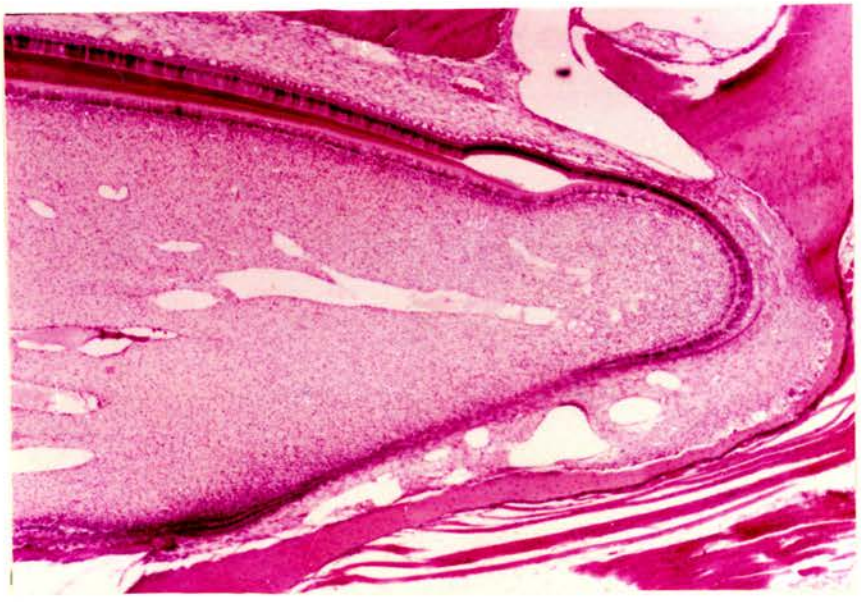

Fig. 66.

L.S. through base of pulp of mandibular right incisor (in occlusion), control; H.& E., X 20.

Toxicity.

T.E.M. is a colourless crystalline solid, readily soluble in water, and stable at low temperature. The lethal dose for mice is 5.0 mgm./kg. intra-peritoneally. Toxic effects involve the haemopoetic system, the liver, lymphoid tissue and the adrenal cortex and thyroid.

Effect on Tissues.

This compound produces marked inhibition of growth of implanted tumour cells in animals and it produces radio-mimetic effects on normal bone marrow and other tissues. It has a mutagenic effect on certain higher bacteria and has a low grade carcinogenic action as its injection into mice is followed by the development of pulmonary adenomata in a large proportion of animals.

Mode of Action.

HENDRY et alia (1951) suggested that the activity of the ethyleneimines depends on the formation of polymer chains and their attachment by means of regularly spaced imine groups to the chromosomes.

Clinical Use.

T.E.M. has been used in avariety of malignant conditions and has produced transient improvement similar to that obtained by nitrogen mustard in, for instance, HODGKINS' disease, lymphosarcoma and the leukemias.

It is generally considered a palliative with temporary effects.

Methods/

Fig. 67.

L.S. through base of pulp of mandibular left incisor (cut out of occlusion), colchicine treated rat; H. & E., X 20.

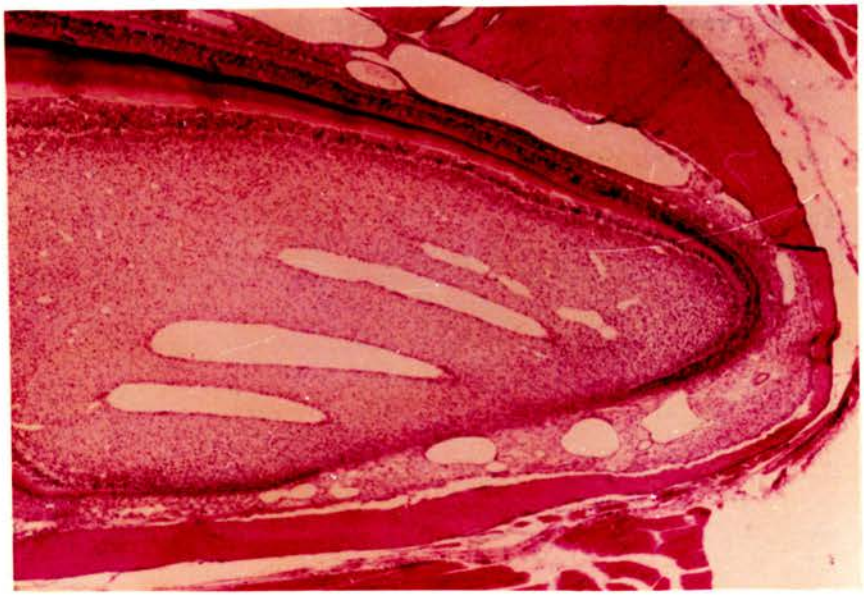

Fig. 68.

L.S. through base of pulp of mandibular right incisor (in occlusion), colchicine treated rat; H. & E., X 20.

Methods.

Six rats, three male and three female, all siblings and three months old, of the strain maintained in the University of Edinburgh were used. Over a period of eight days one pair were injected daily with 1 mgm./kg. of demecolcine intraperitoneally, another pair were injected intraperitoneally with triethylene melamine in doses rising from 0.15 mgm./kg. to 0.6 mgm./kg., and the third pair were injected intraperitoneally with 0.20 mls. water.

The drugs were made up so that the volume injected daily was always 0.20 mls..

On every second day, the left mandibular incisor was cut back out of occlusion and the animals were weighed.

On the tenth day the animals were given the usual drug injection and one from each pair of rats, the female, was then given 200 micro-curies tritiated thymidine by subcutaneous injection at 9.30 a.m.. On the same day, between 3.30 p.m. and 4 p.m., all the animals were killed by an overdose of ether, perfused with 10% formalin, the mandible and tongues dissected out, and placed in 10% formalin for 2 days.

They were processed in the usual manner for histological examination and both halves of each mandible sectioned at a setting of 6 microns, sagittally.

Some sections were stained with haematoxylin and eosin. Some sections from the animals which had been given the radioactive isotope had Kodak stripping film (A.R.10) placed over the tissue and were then placed in light-tight boxes to expose. The optimum exposure time was found to be 8 weeks, after which the film was developed and the tissue stained through the film with haematoxylin alone.

Results/

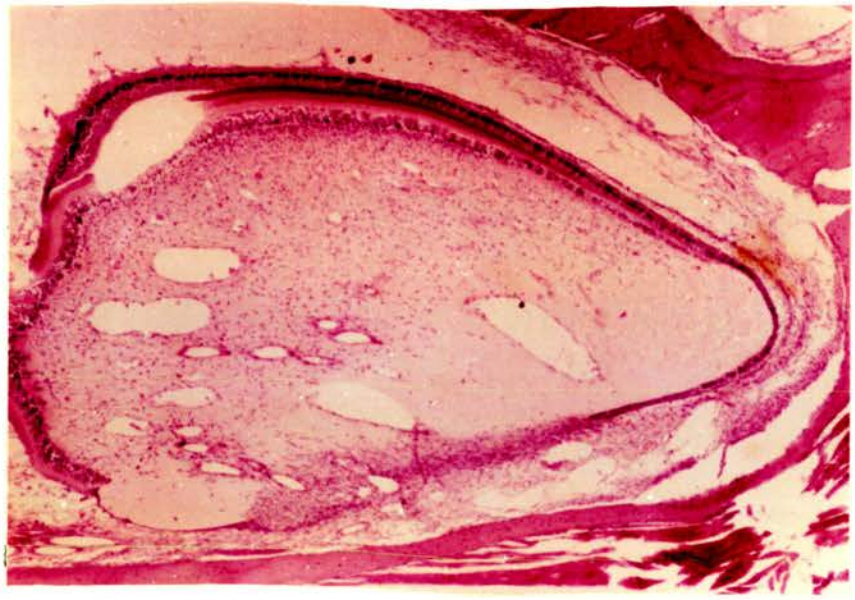

Fig. 69.

L.S. through base of pulp of mandibular left incisor (cut out of occlusion), T.E.M. treated rat; H. & E., X 20.

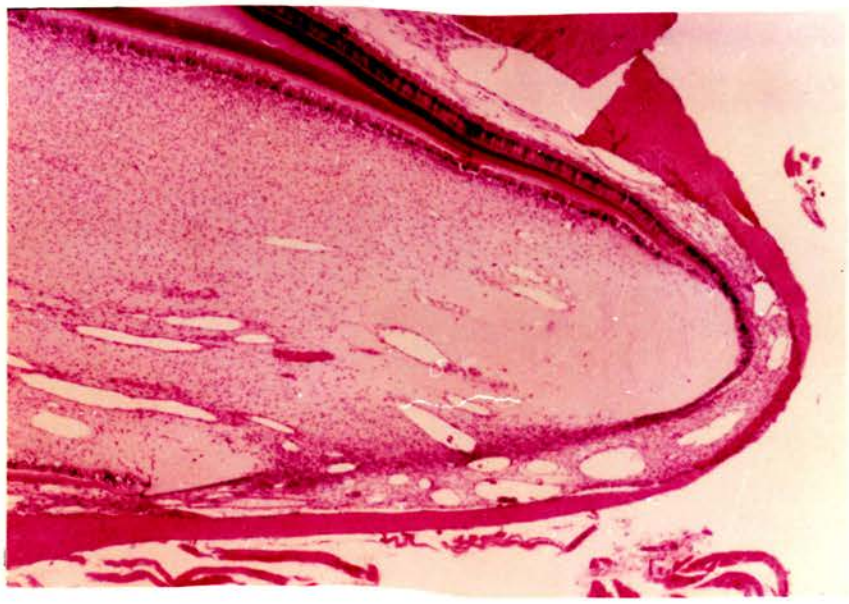

Fig. 70.

L.S. through base of pulp of mandibular right incisor (in occlusion), T.E.M. treated rat, H. & E., X 20.

Results.

The control animals remained healthy throughout the course of the experiment. Their weight did not vary more than plus or minus 5 gms. which was the limit of accuracy of the balance used.

The animals receiving demecolcine developed mild diarrhoea, but otherwise were healthy and clean. Their weight did not vary more than plus or minus 5 gms..

The animals receiving T.E.M. lost weight fairly rapidly, their weights falling from 235 to 205 gms. and from 220 to 200 gms. respectively. They looked ill and their fur became mangy and ill kempt. They both suffered from severe diarrhoea.

Microscopic Results.

To enable comparison to be made, the sections were cut through the open apex of the incisor in all cases. This is not an easy manoeuvre in the rat due to the fairly marked spiral of the mandibular incisor.

Photomicrographs of representative sections of all animals are shown in Figs. 65 - 70. It will be noticed that there is a greater cell density in the pulp of the left (cut) incisor than in the pulp of the right incisor and this can be seen in both the control animal and the animal which had had colchicine.

At this magnification (X 20) it was not possible to distinguish between the control and colchicine treated rats. However, at higher magnification (X 200) in Figs. 71 - 76, large numbers of pulp cells in metaphase can be seen in the colchicine treated animal.

While/

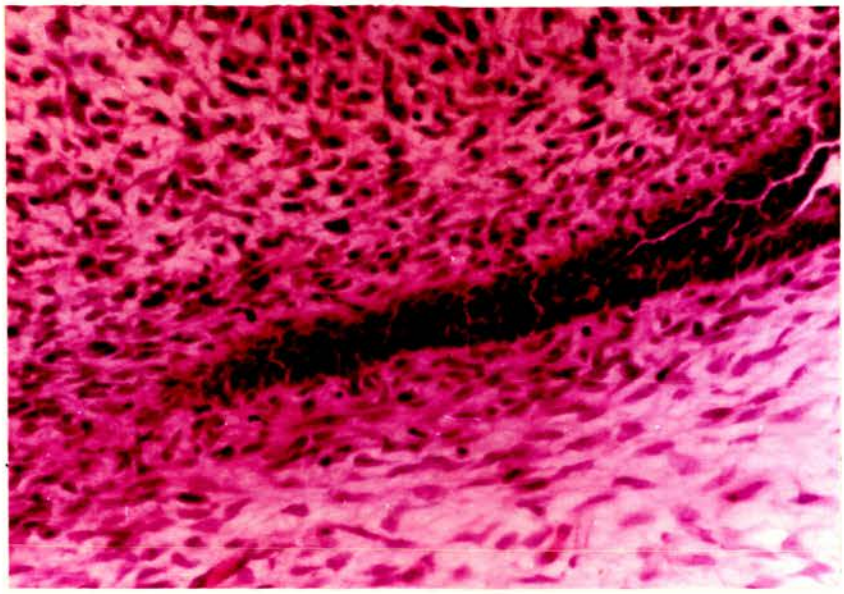

Fig. 71.

Enamel organ, pulp and periapical tissue of control rat; left mandibular incisor; H. & E., X 200.

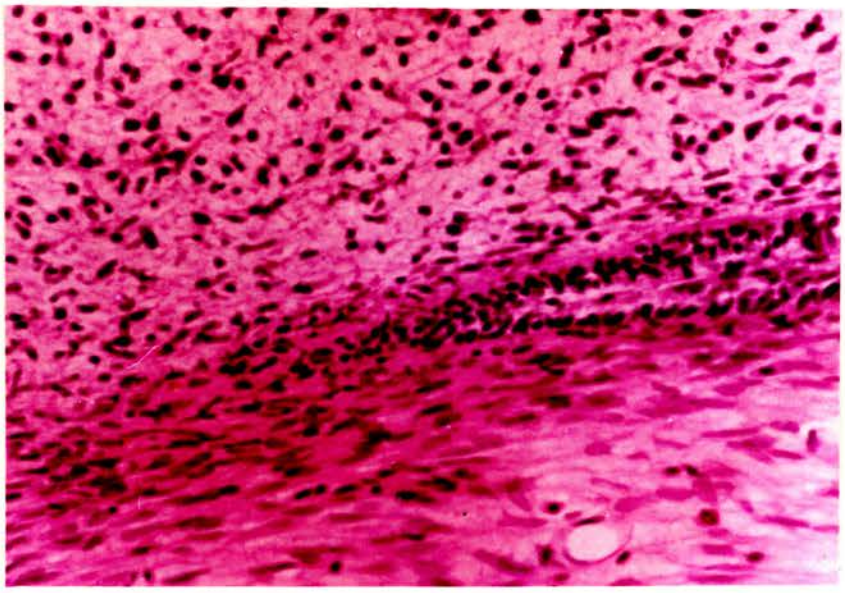

Fig. 72.

Enamel organ, pulp and periapical tissue of control rat; right mandibular incisor; H. & E., X 200.

While in the control animals numerous mitotic figures are visible, there is not the preponderance of cells stopped in metaphase. This preponderance of cells in metaphase is a characteristic effect of colchicine.

The most striking changes were seen in the T.E.M. treated rats, where the effect of the drug had been to interfere with the process of mitosis t such an extent that almost no new cells at all were produced in the proliferative region of the pulp adjacent to the enamel organ and sheath of HERTWIG.

The enamel organ and sheath of HERTWIG themselves had been severely damaged and the ameloblasts and odontoblasts most recently formed were obviously abnormal. Dentinogenesis had been disturbed and there were cellular inclusion in the dentine (Fig. 69). The fibrous tissue of the periodontal membrane was less affected than the pulp, although it was affected to some extent, being less dense and less cellular than in the control animals.

Autoradiographs.

The radioactive index and the total cell density were counted in 10 sections from each side of each rat by means of a squared graticule in the microscope eye-piece at a magnification of times 480.

Because of the variations in the angle of cutting, it was impossible to count exactly comparable areas on each section, but to reduce this sourc of variation to a minimum, the areas where the counts were made were square which/

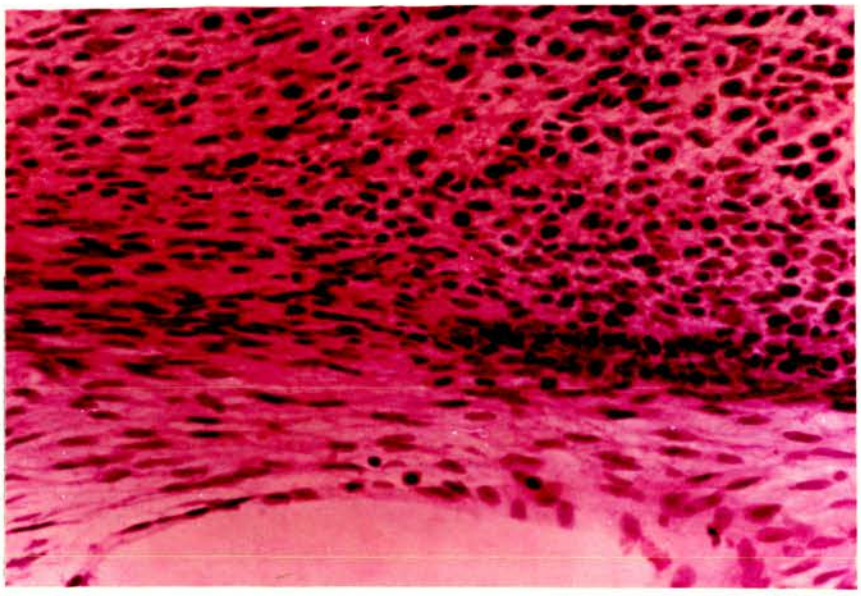

Fig. 73.

Enamel organ, pulp and periapical tissue of colchicine treated rat, left mandibular incisor; H.& E., X 200.

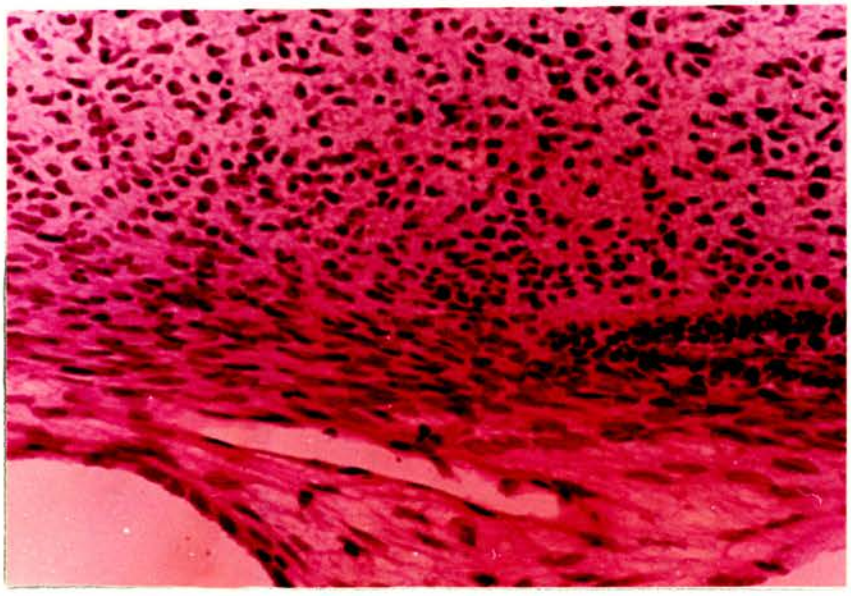

Fig. 74.

Enamel organ, pulp and periapical tissue of colchicine treated rat, right mandibular incisor; H.& E., X 200.

which had their base 1) parallel to the descending limb of the enamel organ and 2) parallel to the ascending limb of the sheath of HERTWIG.

In practice, the figures obtained from these counts did not provide any more information than could be deduced from visual examination of the stained sections, and autoradiographs. They confirmed that there was an increased cell density in the pulp of the cut incisor and that the radioactive index was also higher in this tooth when compared to its neighbour. (Figs. 77 - 82).

There was no difference in radioactive index or cell density between the control and colchicine treated animals - which is again fairly obvious from comparison of Figs. 65, 66 and 67, 68.

That the cell density was almost zero in these areas in the T.E.M. treated animals is obvious.

The distribution of the radioactivity showed that there were two proliferative areas of the pulp in the normally erupting rat incisor. One of these was enclosed by the hook of the enamel organ, the other was the area immediately subjacent to HERTWIG's sheath. Between these two areas, the neurovascular bundle enters the pulp and there are relatively few cells elaborating D.N.A.. These areas are best seen in Figs. 77, 78 and 79, 80.

Radioactivity was present throughout the rest of the pulp but to a much lesser degree, and many of the labelled cells were in the walls of blood vessels.

In/

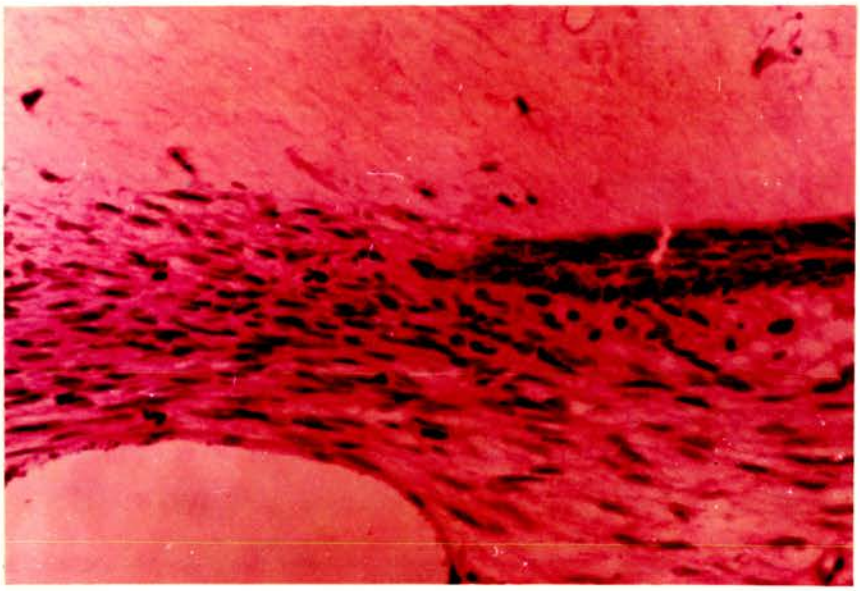

Fig. 75.
Enamel organ, pulp and periapical tissue of T.E.M. treated rat, left mandibular incisor; H.& E., X 200.

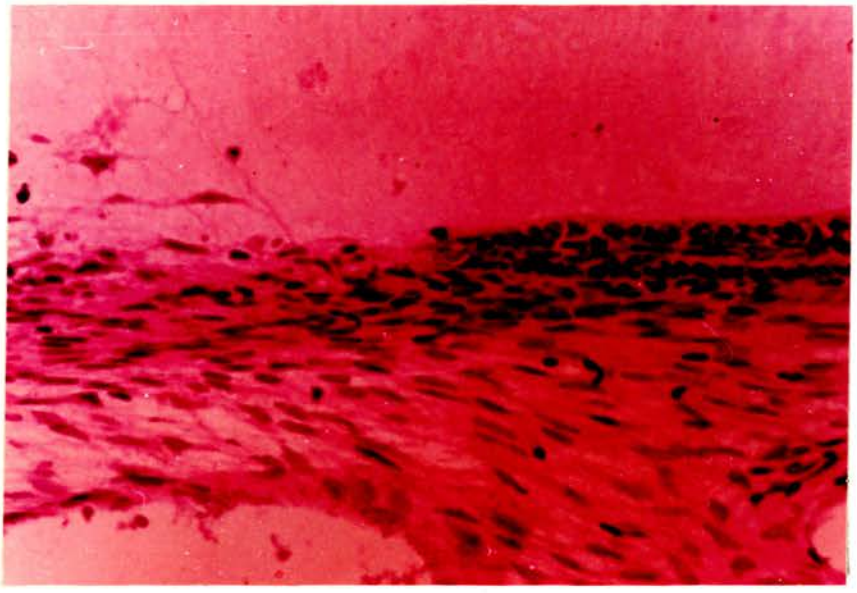

Fig. 76.
Enamel organ, pulp and periapical tissue of T.E.M. treated rat, right mandibular incisor; H.& E., X 200.

In the teeth which have been cut out of occlusion, the distribution of the labelled cells was much wider than in the occluding teeth. The area where the radioactive index was high covered the entire base of the pulp and continued further up the pulp (Figs. 77, 79).

Counts were also done along the full length of the periodontal membran on both the cementum and the enamel sides. These again showed no differen between control and colchicine treated animals and a considerable reduction in radioactive index in the T.E.M. treated animals, but the reduction was much less than in the pulp.

The distribution of radioactive cells in the periodontal membrane was fairly constant along its whole length from the beginning of dentine format Most of the labelling was in the part of the periodontal membrane close to the cementum.

Discussion.

Autoradiography.

The investigation of the distribution of mitoses has only been possibl until recently through direct microscopic observation of cells undergoing this process, and, in mammalian cells, this is difficult and tedious.

The method used in this experiment was introduced by C.P. LEBLOND and his colleagues and its accuracy and specificity has been demonstrated by th and generally accepted (AMANO et al., 1959).

Briefly, the method is to inject thymidine labelled with tritium, sacrifice the animal, prepare sections by the usual histological methods an then demonstrate the position of the labelled thymidine by autoradiography.

Thymidine/

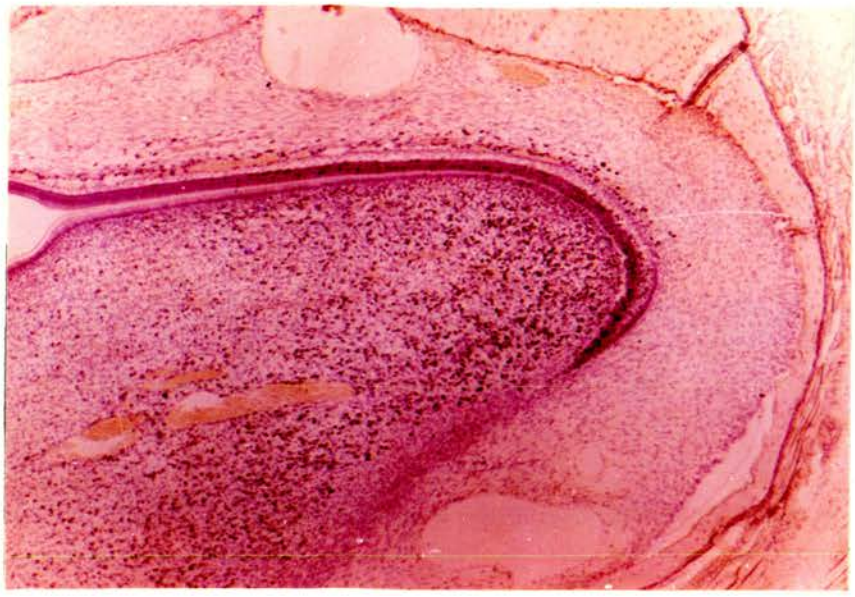

Fig. 77.
Autoradiograph of left incisor, control rat;
stained haematoxylin; X 32.

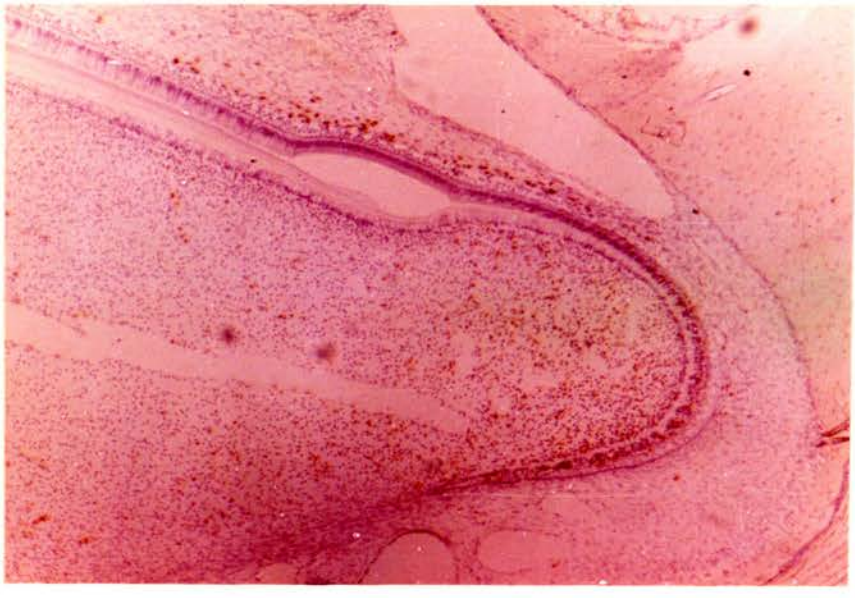

Fig. 78.
Autoradiograph of right incisor, control rat;
stained haematoxylin; X 32.

Thymidine is one of the four constituent nucleosides of desoxyribonucleic acid. It has been shown that cells elaborate D.N.A. only for a short period prior to undergoing mitosis. When thymidine is given by injection, it is utilised by those cells elaborating D.N.A. but by no others.

Tritium is an electron-emitting isotope and, as the electrons have a short penetrating power, (1-3 microns for the majority, with a maximum of 5-6 microns) excellent localisation of the isotope is possible by autoradiography.

The ratio of labelled cells to total cells is called the radioactive index and this is closely related to the mitotic index. For purposes of comparison in a study such as this, they are interchangeable.

Control Animals.

The observation of increased cell density and higher radioactive index in the pulp of the tooth cut out of occlusion is in agreement with the findings of NESS & SMALE (1959), although they found that in the most basal millimetre of the pulp of a rapidly erupting rabbit incisor the cell density was lower than normal. Further incisally, it was higher. This does not appear to be the case in the rat (Figs. 77, 79).

The distribution of mitoses in the periodontal membrane was the same as that found by NESS & SMALE (1959) in the rabbit.

Colchicine/

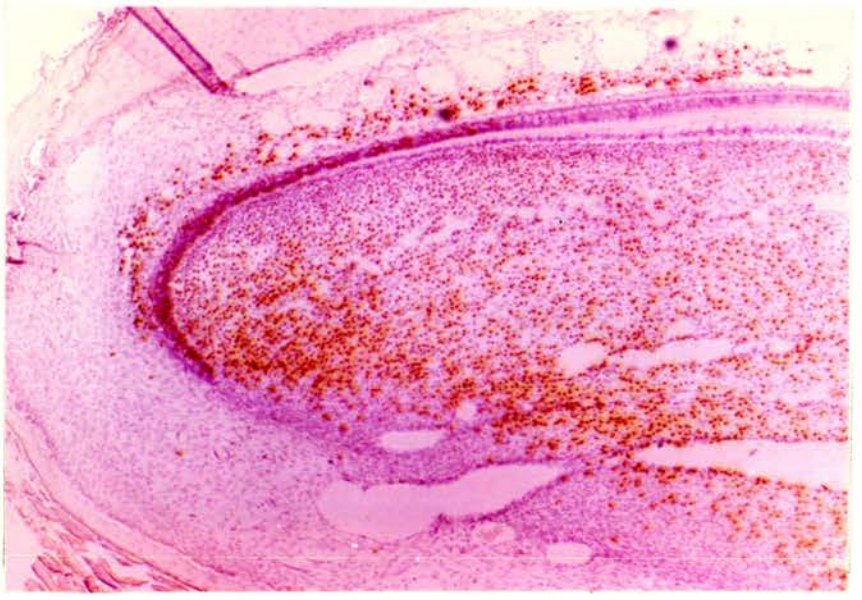

Fig. 79.
Autoradiograph of left incisor, colchicine treated rat; stained haematoxylin, X 32.

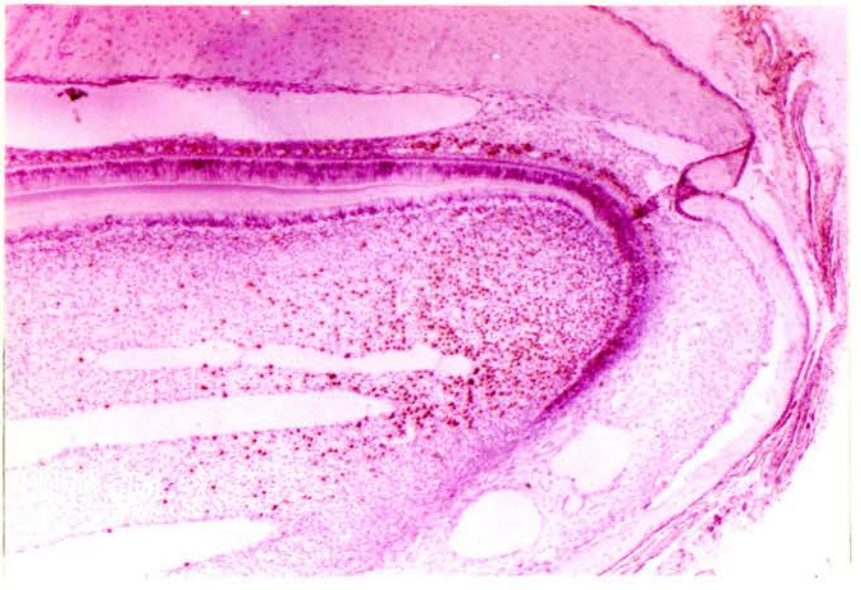

Fig. 80.
Autoradiograph of right incisor, colchicine treated rat; stained haematoxylin, X 32.

Colchicine-treated Animals.

From the observations that the cell density did not decrease in these animals, it could be deduced that either 1) the rate of eruption of the tooth was slower than in control animals or 2) the cells were managing to "escape" from colchicine blockage of mitosis, and complete the process of division or 3) cellular proliferation had no relationship to rate of eruption.

As the rate of eruption was not known, these three possibilities could not be resolved.

The observation that the radioactive index did not rise under the influence of colchicine is difficult to explain, in view of the fact that direct microscopy of the pulp cells (Figs. 73, 74) showed that the typical colchicine affected mitotic figures were present.

Even if the cells were "escaping" from the retarding effects of the drug, the radioactive index should rise from control values in animals killed six hours after administration of the drug and the tritiated thymid

The inability to demonstrate this may be due to a defect in experimental technique in which areas not strictly analagous were being compared.

However, this experiment did demonstrate that colchicine had an effec on the cellular turnover of the dental pulp, although what this effect was remained obscure.

T.E.M./

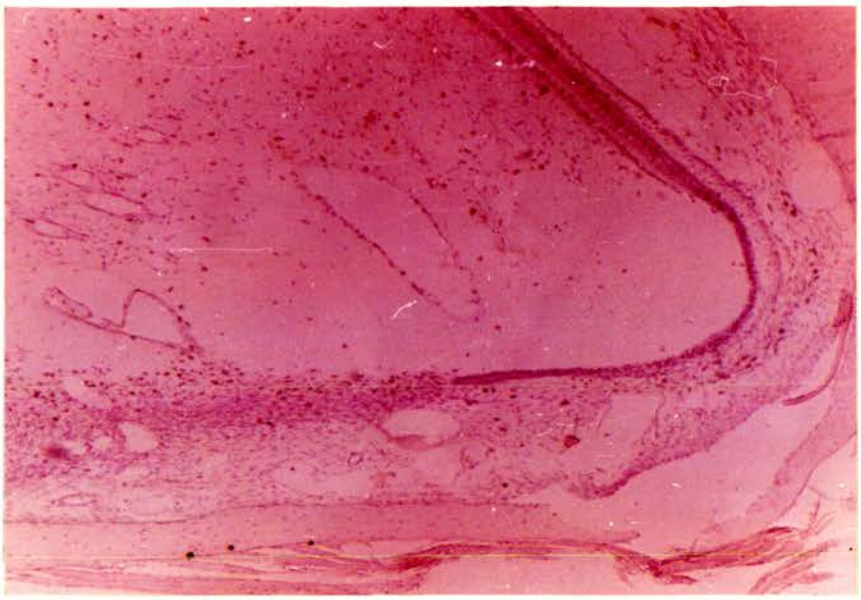

Fig. 81.
Autoradiograph of left incisor, T.E.M. treated rat; stained haematoxylin; X 32.

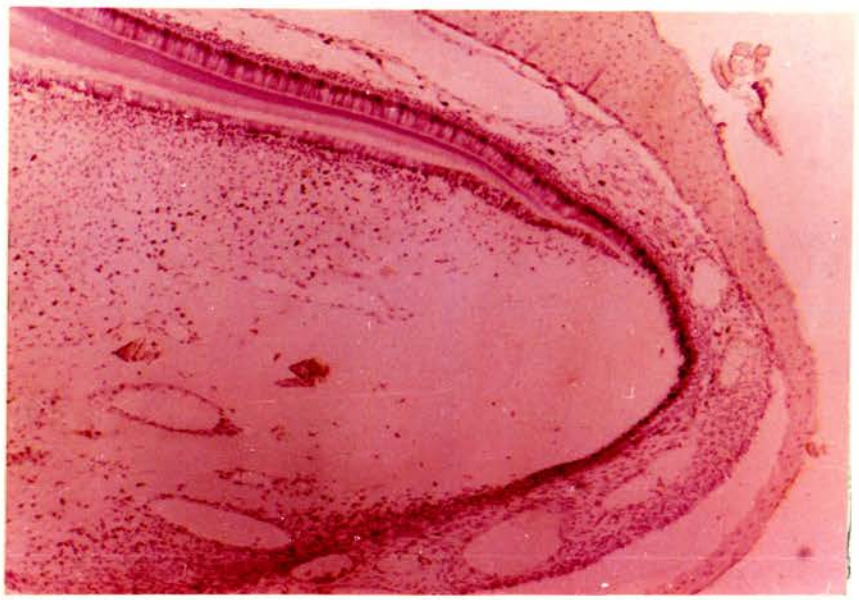

Fig. 82.
Autoradiograph of right incisor, T.E.M. treated rat; stained haematoxylin; X 32.

T.E.M. treated Animals.

T.E.M. affects the dental pulp specifically and selectively, as might have been supposed from a knowledge of its pharmacological action generally.

In the dosages used, it almost completely stops cell division in the dental pulp. It also reduces cell division in the periodontal membrane, although it does not stop it completely.

As a result of the effects on the pulp and enamel epithelium, the enamel and dentine produced after its administration is thin and poor in quality.

Examination of the autoradiographs of the T.E.M. treated animals reveals that a very high percentage of the remaining viable cells in the basal part of the pulp are labelled, and this is consistent with the proposition that T.E.M. does not stop D.N.A. production (HENDRY et al. 1951).

From the observation that a space is present in the base of the pulp of animals treated with T.E.M., it can be deduced that either 1) eruption has continued while pulpal cell proliferation has failed to maintain complete cellularity of the part, or 2) eruption has stopped, while the T.E.M. has been directly toxic to the pulp cells producing their total destruction.

Conclusion.

Demecolcine and triethylene melamine were suitable drugs to use in an investigation of the cellular proliferation theory of tooth eruption as both had specific actions on the dental pulp and periodontium.

EXPERIMENT VI.

Object.

To measure the effects on the rate of eruption of the rat incisor of guanethidine and hydralazine, and demecolcine and triethylene melamine.

Introduction.

This series of eruption measurements really contained two experiments, the first being a repeat of Experiment III using the radiographic measurement method. The second was an attempt to investigate the theory of eruption put forward originally by SICHER (1942a) when he suggested that the eruptive movement was the result of pressure produced by cellular proliferation in the pulp. It has been shown in Experiment V that triethylene melamine practically stops all cell division in the dental pulp of the rat incisor in some manner and that demecolcine affects mitosis in the rat incisor pulp but does not stop it. If the rate of eruption of the rat incisor is governed entirely by the pressure produced by pulp cell proliferation, then this rate should be affected to some extent by demecolcine and stopped completely by triethylene melamine.

Material and Methods.

Thirty albino rats of the same strain as those used in the previous experiments were obtained. Fifteen were male, fifteen female and all were approximately three months old. The number was made up of rats from as few litters as possible.

They/

They were caged in groups of six by sex and were fed stock rat cake and water ad libitum. In addition they were given whole meal brown bread.

The rate of eruption of the left mandibular incisor was measured using the technique described in the introduction to Experiment IV, for a period of 24 days. They were weighed at the same time as eruption measurements were made.

The animals were divided into five groups of three males and three females and from the eighth through the fifteenth day they were given daily injections as shown in table IV.

TABLE IV.

Day	8	9	10	11	12	13	14	15
Group I Water.				0.20 mls.				
Group II Guanethidine			10 mgm./kg.			15 mgm./kg.		20 mgm./kg.
Group III Hydralazine	4 mgm./kg.		6 mgm./kg.		10 mgm./kg.		15 mgm./kg.	
Group IV Demecolcine				1 mgm./kg.				
Group V T.E.M.	0.15mgm./kg.		0.3mgm./kg.		0.4 mgm./kg.		0.6 mgm./kg.	

The/

The sterile water, guanethidine and hydralazine were given by intramuscular injection into the hind leg. The demecolcine and triethylene melamine were injected intraperitoneally.

The radiographs were developed and mounted on glass slides for projection as they were taken, but the measurements were not made until the experiment was completed. Measurements were made from the incisal crest of the alveolar bone surrounding the uncut incisor.

Drugs.

The drugs used, guanethidine, hydralazine, demecolcine and triethylene melamine have all been described in the accounts of the previous experiments.

Results.

The angulation of the casette was slightly different in this experiment from what it was in Experiment IV C and, instead of working out an individual figure of radiographic distortion for each eruption increment, an average value of 12% was used for all measurements. This figure was obtained in the same way as in Experiment IV C.

The individual measurements with this correction are in appendix 6,A.

Three animals died due to an accidental overdosage of ether during the experiment, BM2, CM2 and CF1.

All animals which had been given triethylene melamine died in the period subsequent to stopping the drug. It will be seen from the weights of the animals in appendix 6,B, that the T.E.M. group began to lose weight by the fourth day of drug administration and continued to do so until death.

This/

This loss of weight produced obvious emaciation and the animals also suffered from diarrhoea after the sixth day of drug administration. These animals became very stiff when handled, as if they had lost their normal tissue elasticity. In two instances they were obviously moribund when measurements were being made and they were intentionally killed by an overdose of ether. In both cases marked post-mortem rigidity developed immediately.

All other groups remained healthy throughout the course of the experiment and continued to increase in weight slowly. The weighings were made on a BUTCHART balance with lever arm and counterpoise for speed, and the weight was taken to the nearest 5 gms.. Again a small number of weights are missing due to the rat recovering consciousness before the weighing was completed. It was considered unnecessary to re-anaesthetise the animal only to weigh it.

Analysis of the Results.

A goodness of fit test to a normal distribution was applied to the pooled control observations and a histogram of these made. (Appendix No. 6,C and Fig. 83). Chi-square was calculated to be 6.45.

The five per cent probability limit for the relevant degrees of freedom is 12.59, therefore the usual tests for significance of differences between means are appropriate.

The pooled mean for all control observations was 1.91 m.m./48 hours with a standard deviation of 0.17.

The/

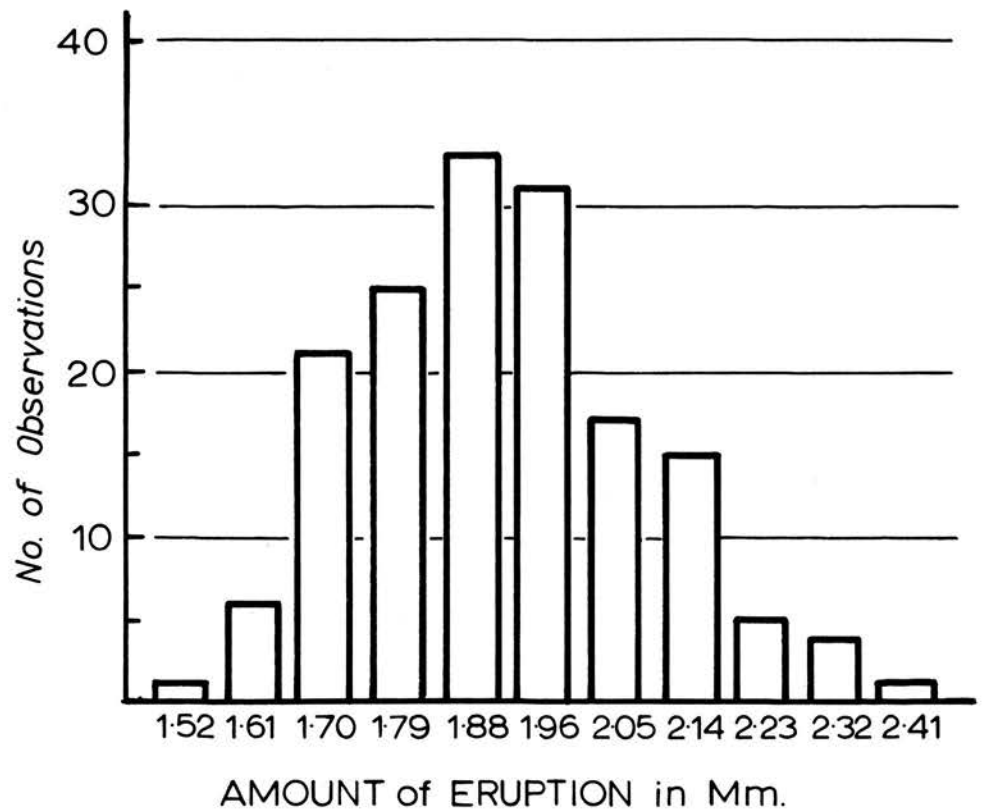
40
30
20
10
No. of Observations
1·52 1·61 1·70 1·79 1·88 1·96 2·05 2·14 2·23 2·32 2·41
AMOUNT of ERUPTION in Mm.

Fig. 83.

The means and standard deviations of the rate of eruption of the left mandibular incisor of all groups for the initial control period, the period of drug administration and the second control period were calculated and are shown in Table No. V.

The pooled mean eruption increments for each group for each 48 hour period were also calculated and these have been graphed in Figs. 84, 85.

TABLE V.

	1st. Control Period	Period of Drug Administration	2nd. Control Period
Group I Control	$\bar{x}$ = 1.90 s = 0.15 n = 24	$\bar{x}$ = 1.88 s = 0.14 n = 23	$\bar{x}$ = 1.95 s = 0.19 n = 18
Group II Guanethidine	$\bar{x}$ = 1.91 s = 0.20 n = 24	$\bar{x}$ = 1.88 s = 0.19 n = 24	$\bar{x}$ = 1.94 s = 0.19 n = 24
Group III Hydralazine	$\bar{x}$ = 2.01 s = 0.11 n = 24	$\bar{x}$ = 1.97 s = 0.25 n = 24	$\bar{x}$ = 1.95 s = 0.23 n = 24
Group IV Demecolcine	$\bar{x}$ = 1.86 s = 0.18 n = 23	$\bar{x}$ = 1.86 s = 0.25 n = 23	$\bar{x}$ = 1.99 s = 0.21 n = 20
Group V T.E.M.	$\bar{x}$ = 1.90 s = 0.15 n = 23	$\bar{x}$ = 1.54 s = 0.44 n = 24	$\bar{x}$ = 0.43 s = 0.30 n = 9

Means ($\bar{x}$), in millimeters per 48 hours, standard deviations (s) and numbers of observations (n) of rates of eruption.

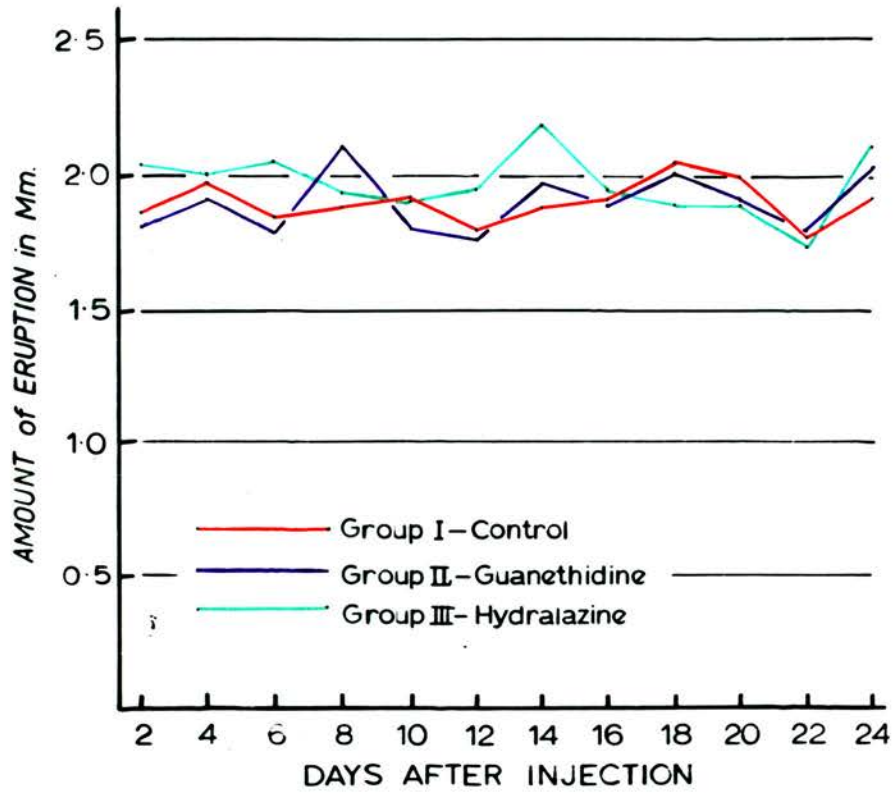
2·5
2·0
1·5
1·0
0·5
AMOUNT of ERUPTION in Mm.
Group I – Control
Group II – Guanethidine
Group III – Hydralazine
2 4 6 8 10 12 14 16 18 20 22 24
DAYS AFTER INJECTION

Fig. 84.

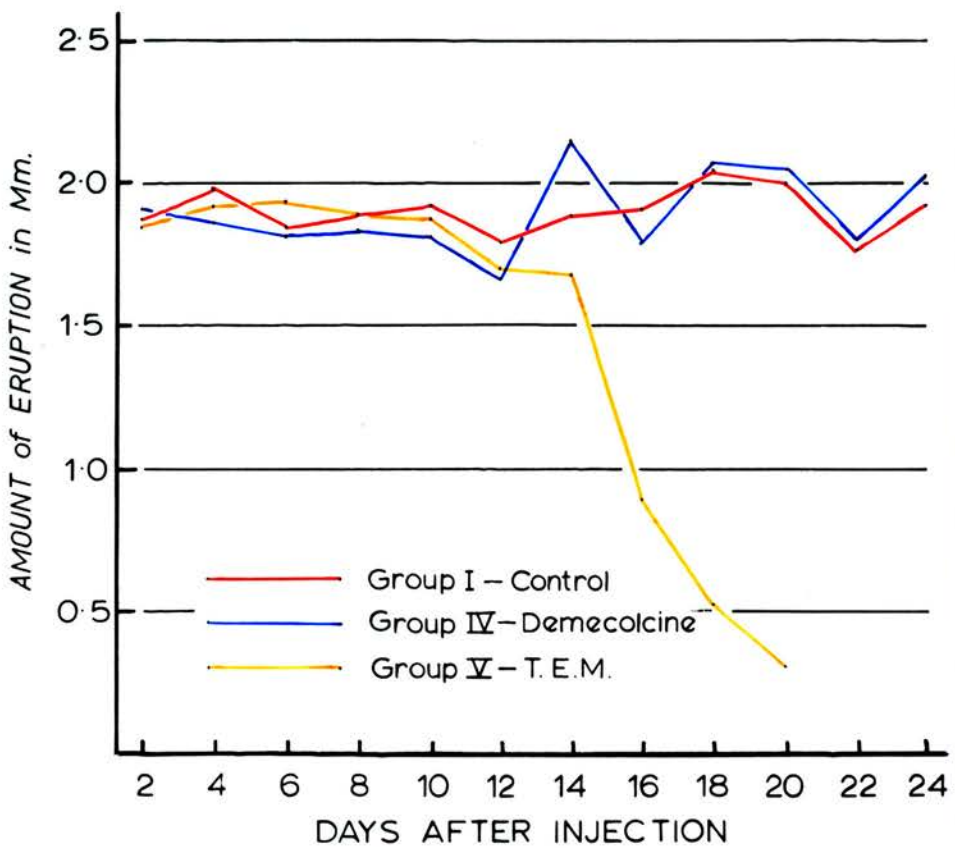
2·5
2·0
1·5
1·0
0·5
AMOUNT of ERUPTION in Mm.
Group I – Control
Group IV – Demecolcine
Group V – T. E. M.
2 4 6 8 10 12 14 16 18 20 22 24
DAYS AFTER INJECTION

Fig. 85.

Tests for Significance of Differences Between Means.

1. Between groups during initial control period.

Tests were applied between the means of each group, and these showed that the only difference which was significant at p. less than 0.100 was between the mean of group III and the mean of the other groups. There was also a significant difference between the mean of group III and the pooled mean for all groups.

e.g. between group III and group I

$$S_{\bar{x}_1 - \bar{x}_2} = \sqrt{\frac{S.D.^2}{n_1} + \frac{S.D._2^2}{n_2}}$$

$$= \sqrt{\frac{0.11^2}{24} + \frac{0.15^2}{24}} = \underline{0.04.}$$

$$t = \frac{\bar{x}_1 - \bar{x}_2}{s_{\bar{x}_1 - \bar{x}_2}}$$

$$= \frac{2.01 - 1.90}{0.04} = \underline{2.75.}$$

$$D.f. = (n_1 - 1) + (n_2 - 1) = 46.$$

From the t. tables, we find p is less than 0.010. In other words, this difference could have arisen by chance in less than one instance in a hundred.

2./

2. Between means of the same group before and during drug administration.

t. tests applied to the difference between the means before and during drug administration showed that the only significant difference is to be found in group V where the reduction in rate of eruption is very obvious.

3. Between means of the same group during and after drug administration.

No significant differences can be found between means of groups I, II or III. The difference between the means in group V is significant. The difference between the means in group IV, while apparently fairly big, is not significant.

A preliminary report of this experiment was given to the British Division of the International Association for Dental Research in April, 1962, and an abstract of the paper published afterwards. (Main, J.H.P., and Adams, D. Eruption Studies on the rat incisor. J.dent.Res., 41, 1249, 1962).

DISCUSSION.

Distribution of Control Observation.

It will be seen that the value for chi-square in the goodness of fit test is higher than that obtained in Experiment IV C, 6.45 as opposed to 3.29. Further, there can be little doubt that the difference between the pooled mean of group III and the pooled means of the other groups reflects a real difference in the rate at which the incisors were erupting. Therefore there has been considerable animal variation present, which is one reason for the higher value of chi-square.

Another reason for the highness of the value of chi-square is that a flat rate of correction for radiographic distortion was used in this experiment while in Experiment IV C individual corrections were calculated.

However, tests for significance of difference between means of normally distributed observations are obviously valid.

Rate of Eruption - Normal, Unimpeded Values.

The pooled mean rate of eruption was 1.91 m.m./48 hours, standard deviation 0.17 m.m.. This is significantly different (p less than 0.010) from the mean found in Experiment IV C for a group of animals of the same line-bred strain. This is probably due to the animals in this experiment being a few weeks younger than those used in Experiment IV C as it is a widely held belief among workers in this field that the rate of eruption of continuously erupting teeth decreases with age, although no one has published any direct evidence of this.

The/

The Effect of Reducing Arterial Blood Pressure on Eruption Rate.

As was shown in Experiments I and II, the results of the administration of guanethidine and hydralazine to rats are to lower the arterial blood pressure and to raise the mean small vessel pressure. If CONSTANT's theory that tooth eruption is the result of pressure being transmitted from the blood vessels to the inside of the pulp chamber is correct, then in the continuously erupting rat incisor the result of administration of hypotensive drugs would be:-

(a) an increase in the rate of eruption if the pressure was coming from the small vessels.

(b) a decrease, if the pressure was being transmitted from the arteries and arterioles.

It has been conclusively shown that neither of these possibilities actually occur, the rate of eruption being unchanged, and therefore the original theory must be untrue.

The Effect of Demecolcine and Triethylene - Melamine on Eruption Rate.

The administration of demecolcine had no effect on the rate of eruption.

The administration of triethylene melamine produced no detectable effect during the first 48 hours. Subsequently the rate of eruption was progressively depressed, and this deceleration continued after withdrawal of the drug. However, in no case was the eruption of the incisor actually stopped.

The significance of these findings will be discussed in the general discussion.

General Discussion.

The Problem.

To begin with it is obviously desirable to have a clear definition of the problems involved in attempting to understand the process of eruption. It seems reasonable to accept Orban's (1928) concept of the relative fixity of position of the sheath or, more accurately, diaphragm of Hertwig, as a generalisation.

What is really known about this is that in no case which has been investigated has there ever been found any evidence of bone resorption occurring in the base of the alveolus of an erupting tooth and, conversely, the epithelial diaphragm has never been found to move in an occlusal direction. The evidence in favour of Orban's concept has been reviewed.

The exception to the rule of stability of relative position of the epithelial diaphragm is found in young animals with continuously erupting incisors, as has been described previously. In young rats there is very active resorption of the base of the alveolus of the incisor, and the position of the apex of the incisor is just below the molar teeth. In adult rats, the apex lies high in the vertical ramus of the mandible. The finding of rapid resorption of the base of the alveolus shows that the change in position of the apex is produced by a backward growth of the tooth, and not by forward growth of bone to any great extent.

It/

It is remarkable that, once having attained the adult position, the position of the apex becomes stable, covered on the lateral aspect of the ramus by only a very thin layer of compact bone.

The presence of osteoclasts in the base of the alveoli of incisors in young rabbits, mice and guinea pigs, indicates that a similar ingrowth of the tooth occurs, although in these animals the posterior movement of the apex of the tooth is much less.

However, with the exception of this special case of continuously erupting incisors in young animals, no instances have been found of resorption in the base of the alveolus of an erupting tooth.

Accepting the stability of the epithelial diaphragm then, we have to account for the movement of the tooth from its formative position in the bone to its adult position in the mouth. The crown must move first through the bone of the alveolar process which Brash has shown to be itself growing in the same direction. It has to penetrate the oral mucous membrane and move further away from the alveolar process until the crown is exposed to the requisite functional length.

The Role of the Periodontal Membrane.

During this period of rapid relative movement - the phase known as "active eruption" - the tooth is firmly anchored at all times to the bone of the alveolus by the periodontal membrane.

It seems reasonable to accept that the period of active eruption in a tooth of limited growth is exactly comparable to the eruption of a tooth of continuous eruption. This evidence in favour of this concept has been reviewed (page 12).

It/

It is known that there is a gradient of velocity across the periodontal membrane in such continuously erupting teeth (NESS & SMALE, 1959) and therefore Brash's concept of bone deposition carrying tooth and periodontal membrane toward the mouth must be discarded.

Therefore there must be a process whereby the periodontal membrane allows movement of the tooth relative to the bone while continuously and simultaneously binding the tooth to the bone. It was suggested by SICHER (1942a) that this adjustment takes place in the intermediate plexus and this postulate has been generally accepted. However, there have been few investigations of the processes involved. NESS & SMALE (1959) described the distribution of mitotic figures along the periodontal membrane in the rabbit. ECCLES (1962) found that there is no increased turnover of sulphate containing muco-polysaccharide in the intermediate plexus as compared to the other parts of the periodontal membrane, although this observation needs replication.

It seems certain that further information on the process by which the periodontal membrane allows the tooth to move while also attaching it to the bone will be of basic importance to understanding eruption.

Eruption and Proliferation of Pulp Cells.

But to return to the main point, the question which has been asked for the last hundred years or so is by what means does the tooth move? Now it can be suggested that this is merely a matter of differential growth, that is to say a rapid proliferation rate of the odontogenic cells in the base of the pulp/

pulp with a slower proliferation rate of the tissues overlying the teeth in the case of completely embedded teeth, while in those teeth in which the crown has penetrated the mucosa there is no cellular pressure in the opposite direction. This postulate presupposes that the small pressures produced by the cellular proliferation are sufficient to cause the periodontal membrane to allow the tooth to move past it - while retaining its own structural integrity.

A stumbling block to this theory has been the total absence of any evidence of resorption of bone in the base of the alveolus, as it has been presumed that cellular pressures sufficient to cause axial movement of the teeth would also be sufficient to cause some bone resorption in the base of the crypt. The existence of the hammock ligament resolved this difficulty for SICHER (1942a & b) and SCOTT (1953). But in this work, and in the work of NESS & SMALE (1959), HUNT (1959) and ECCLES (1961), the hammock ligament has been found not to exist.

Further evidence against this theory is found in the work of HERZBERG & SCHOUR (1941a) who removed the pulp from the rat incisor and found that eruption continued, and in the confirmation of these findings by KOSTLAN et al (1960). Again, TAYLOR & BUTCHER (1951) found that eruption continued in spite of complete pulpal necrosis in the rat incisor, and GOWGIEL's finding that eruption continued in the monkey when odontogenesis has been completely inhibited by radiation, is further evidence against the proliferation of odontogenic cells having a causal role in tooth eruption.

It/

It is suggested that the findings in Experiment VI of this work taken in conjunction with the work of the above writers, prove conclusively that the proliferation of pulp tissue is unrelated to the process of eruption.

It was shown in Experiment V that demecolcine interfered with mitotic activity in the dental pulp in some undetermined manner. And yet in spite of this anti-mitotic activity, the eruption of the tooth was entirely unaffected. Again in Experiment V, it was shown that triethylene melamine stops the successful completion of mitosis in the dental pulp almost entirely - and yet, in rats given this drug in toxic quantities, eruption continued until death, albeit at a much reduced rate. The presence of actual spaces in the pulps of the teeth of these rats proves conclusively that the movement of these teeth cannot be the result of pressures produced by cellular proliferation.

It can be concluded then that the eruption of teeth is not the result of pressure produced by differential rates of cell growth between the dental pulp and the surrounding tissues, unless the axial movement found in the special cases specified above is produced by a different mechanism than exists under physiological conditions. This contingency would seem to be unlikely.

As described in the review of literature, BRYER (1957) carried out a number of experiments in which he extirpated or traumatised the pulp of the rat incisor and observed the effect on the rate of eruption. He suggested that the continued eruption of teeth in which the pulp had been severely damaged/

damaged was the result of blood pressure from the granulation tissue formed as a result of the surgical procedures. While this is a possibility in those cases where pulp necrosis has been produced as a result of surgery, it is not a possible explanation of continued eruption after radiation necrosis as produced by GOWGIEL or in the case of the continued eruption found after the administration of triethylene melamine.

When considering BRYER's work, it is pertinent to remember that throughout his experiments he used a method of measurement with a large intrinsic error of which he gave no indication that he was aware.

Eruption as a Problem in Relative Growth.

This is perhaps a convenient point to consider whether eruption can be considered as an ordinary growth phenomenon, as yet another example of a problem in relative growth. It was stated by SICHER and has been implicit in the writings of many other workers, that eruption is just such a problem of differential growth and surely this was a reasonable basic assumption. However it has not been proved, and it is possible that the phenomenon of eruption lies outwith the biological field of growth, as it is generally known.

As has been argued above, eruption is not related to pulpal growth, nor, as a corollary, is it related to the rate of growth or accretion of the calcified tissue.

One/

One way of showing that eruption could be considered to be an example of relative growth would be to relate it to the growth of the entire animal - and in doing this find that the relationship could be expressed by the simple formula derived by HUXLEY (1932). To put this in another way, it could be asked - is eruption an example of heterogonic growth? If it is, then the relationship of the rate of eruption of a tooth, expressed as the weight of tooth exuded or erupted in unit time, to the rate of growth of the entire animal, again expressed as increase of weight in unit time, should be a simple one.

The general formula for the constant differential growth ratio is $Y = b X^{k}$ (HUXLEY, 1932) where Y is the weight of the differentially growing organ, X is the weight of the entire animal minus the weight of the differentially growing organ, and b and k are constants; b is the fractional coefficient; k is the growth-coefficient.

However the growth of a tooth is an additive or accretionary not a multiplicative phenomenon. Teeth are all parts of logarithmic spirals, as was demonstrated by D'ARCY THOMSON (1961), and the characteristic of such structures is that successive growth increments are all of the same form, though of increasing bulk, this condition being called gnomonic growth.

Therefore the general formula for multiplicative growth does not hold for growth by accretion but is changed from $Y = b X^{k}$ to $Y = b X k$, in other words this type of growth is by simple, not compound interest.

Given/

Given the premise that tooth eruption is an example of heterogonic growth then this formula should be found applicable.

However, the practical difficulties of testing this relationship for any type of accretionary growth are great. It would be possible to do so in the case of continuously growing teeth, if the rates of eruption at a number of ages were known, together with the cross-sectional area and density of the calcified tissues, and also the growth rate of the entire animal. To the writer's knowledge no such examination has been carried out in relation to teeth, or in relation to the growth of any other tissue growing by accretionary means. This is in contrast to the case of multiplicative growth where numerous cases have been examined, all of which can be expressed by the simple HUXLEY formula. As no exceptions to this have been found, the formula is believed to hold good for all cases of heterogonic growth.

The fact that all animals with continuously erupting teeth continue to grow throughout life is indirect evidence in favour of the theory that eruption is agrowth phenomenon, as this permits the possibility of such a relationship between tooth growth and general growth remaining constant throughout the animal's life.

It is suggested that if the relationship of growth of tooth to growth of the entire animal were shown to conform to HUXLEY's formula, then this would be strong, even conclusive, evidence that the phenomenon of tooth eruption is an example of relative growth. Further, the converse should also be true, that is if the above relationship did not conform to the general/

general formula, then this would be strong, presumptive evidence that tooth eruption was a biological phenomenon in its own right, with the implication that the mechanism causing it was different from that causing differential growth.

In the absence of any direct evidence on this question, it is possible that the eruption of a tooth may be a phenomenon unrelated to its own growth, and indeed, most of the available evidence points in this direction.

The relationship between eruption and the endocrine glands.

Thyroid and Pituitary.

The very thorough investigations of BAUME et al. (1954a, b & c) have shown that eruption is not directly affected by pituitary hormones but only through the mediation of the thyroid.

They also showed that while thyroxin stimulates eruption, there is no question of it initiating it.

Adrenal Cortex.

The findings of previous workers on the effects of the adreno-cortical hormones are perhaps the most puzzling of all.

In particular, SOBKOWSKI's clear demonstration of the effects of hydrocortisone on the rate of eruption of rat incisor which have been confirmed by others, including the writer, (unpublished) are difficult to account for. It would seem more likely to expect the directly opposite effect/

effect, that is a retardation of eruption rate, as cortisone is known to depress the rate of mesenchymal cell proliferation, in all instances in which this has been investigated (e.g. CREDITOR et al., 1950; BAKER & WHITAKER, 1950 and others). It has also been shown to depress the rate of nail growth (GODWIN, 1959) which might be expected to be a process similar to eruption.

It may be that the explanation of these curiously contrasting effects of cortisone, reducing nail growth and increasing eruption rate, can be explained on the grounds that cortisone reduces the level of polymerisation of the muco-polysaccharides of connective tissue ground substance (PLOTZ et al, 1950) thereby reducing the retardation of eruption due to the periodontal membrane. Then again, this can be regarded as further evidence against the concept of eruption as an example of a growth phenomenon.

Apart from those discussed above, it would seem that the secretions of the endocrine glands have no direct influence on eruption.

In general terms then, the hormones of the pituitary, thyroid and adrenal cortex have a modifying or controlling action on eruption, but do not cause or initiate it.

Blood Pressure and Eruption.

The blood pressure theory of eruption, although put forward in 1900 by CONSTANT and supported on theoretical grounds by various other writers after that date, e.g. MASSLER & SCHOUR (1941), had little experimental evidence in its favour until the publication of BRYER's work in 1957.

Bryer/

BRYER not only produced a great deal of experimental evidence, all of which, he claimed, supported the blood-pressure theory, but he also introduced a new method of measuring eruption rate in continuously erupting teeth. He claimed that this method reduced the inaccuracies in measurement of eruption rate by avoiding the attritional effects altogether.

As has been explained in the review of the literature, this claim is unjustifiable, although this was not realised by the writer when the experiments reported here were begun. It was further shown in Experiment IV that the variation in length of the uncut incisor, when the other was kept cut out of occlusion, resulted in an intrinsic, undetectable error in BRYER's method which had a range of approximately plus or minus 9%. This finding throws doubt on a number of BRYER's conclusions, particularly the significance of a 5% reduction in eruption rate subsequent to cobalt administration, which was really the key experiment in BRYER's series.

However, disputing some of the findings of one worker does not necessarily disprove the theory which he supported.

Experiments I and II of this work were devoted to proving that guanethidine and hydralazine, both known hypotensive drugs, had a hypotensive effect of some magnitude on normotensive rats, and further, that this arterial hypotension was associated with capillary hypertension. While admitting that the method of measuring capillary pressures was crude, as discussed previously, it seems reasonable to conclude that these blood pressure changes were adequately demonstrated.

Experiment/

Experiment III and part of Experiment VI were then carried out to determine the effects on rate of eruption of these blood pressure changes, and it was found that no effect whatever could be found.

Now this obviously means that there is no direct and intimate relationship between blood pressure, whether capillary or arterial, and rate of eruption.

It does not disprove NESS's (1959) suggestion that the pressure concerned is not blood pressure but the hydrostatic tissue pressure postulated first by STARLING (1909). Indeed, it is possible that the slow eruption found after administration of triethylene melamine in Experiment VI is the result of this hydrostatic pressure.

However it is difficult to accept that tissue hydrostatic pressure can be the sole cause of tooth eruption as it does not satisfactorily account for eruptive movements prior to the crown penetrating the gum, and it does not explain the lack of eruption in cleido-cranial dysostosis, where, as far as is known, the blood vascular system is entirely normal.

Nonetheless in the present state of knowledge, tissue hydrostatic pressure cannot be ruled out entirely as a possible source of pressure in eruption, although if it is accepted as such, additional processes must be postulated in the cases mentioned above.

The process whereby a tooth is enabled to erupt, then, is still speculative, but it is suggested from the present work that pressure from cell proliferation in the dental pulp, any effect attributable to the hammock ligament, and direct pressure from the pressure of blood in large or minute vessels can all be excluded from the process.

On/

On theoretical grounds, it would seem that the mechanism which produces eruptive movements must lie in the periodontal membrane, and elucidation of the problem must await further studies on this tissue.

Conclusions.

From these studies it was concluded that:-

1. The hammock ligament as described by SICHER (1942 a & b) and SCOTT (1953) did not exist. In relation to developing single-rooted teeth of limited growth only, there was found an annular sheet of collagenous fibrous tissue which demarcated the dental pulp from the periapical tissues. This sheet was deficient centrally and attached peripherally to the inner and central zones of the periodontal membrane. It was suggested that an appropriate name for this would be the "pulp limiting membrane". This structure did not exist in multi-rooted teeth of limited growth or in relation to any teeth of persistent growth.

2. A more accurate method of measuring the rate of eruption of the rat mandibular incisor than any previously used has been developed. This method was based on the method devised by NESS (1954 & 1956) for the rabbit. The measurement error of this method was calculated to have a standard deviation of plus or minus 1.15%.

3. One of the effects of keeping one persistently erupting incisor cut back out of occlusion is to increase the rate of bone deposition on the interincisal crest of the alveolar bone of the cut tooth. No effect is produced by this procedure on the interincisal crest of bone of the alveolus of the neighbouring incisor.

4./

4. Guanethidine and hydralazine in the dosages used lowered arterial blood pressure in the normotensive rat.

5. Guanethidine and hydralazine in the dosages used raised minute vessel pressure in the normotensive rat.

6. The administration of guanethidine and hydralazine had no effect on the rate of eruption of the rat mandibular incisor, therefore the rate of eruption of the rat incisor is not directly related to capillary or arterial blood pressure.

7. Demecolcine, in doses of 1 mgm./day intraperitoneally, produced an effect on the process of cell division in the dental pulp of the rat incisor in that the characteristic colchicine induced mitotic figures were produced. However, in some undetermined manner, the pulp cells "escaped" from the retarding effects of the drug.

8. Demecolcine in doses of 1 mgm./day intraperitoneally had no effect on the rate of eruption of the rat mandibular incisor.

9. Triethylene melamine in the dosages used were lethal to the albino rat.

10. Triethylene melamine, in the dosages used, selectively prevented the successful completion of cell division in the pulp of the rat incisor.

11. Triethylene melamine retarded the rate of eruption of the rat incisor markedly, but did not stop it, the result being that spaces developed in the basal end of the pulp of the incisor. Therefore the rate of eruption of the rat incisor is not the result of pressures derived from proliferation of pulpal cells.

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APPENDIX 1.

EXPERIMENT 1.

Normotensive Arterial Blood Pressures in m.m. Hg.

		5 a.m.	10 a.m.	3 p.m.	8 p.m.	12 p.m.
Male	X1	138	144	136	126	142
	X2	130	134	134	126	136
	X3	130	126	136	122	114
	X4	128	128	136	130	128
	X5	118	120	132	130	124
Female	Y1	102	128	104	130	116
	Y2	116	124	132	134	144
	Y3	98	114	108	124	130
	Y4	128	122	130	136	114
	Y5	122	122	118	112	118
Means	-	121.0	126.2	126.6	127.0	126.6
Standard Deviations		12.8	8.2	12.1	7.0	11.3

Mean of all 10 rats = 125.5 m.m. Hg. S.D. = 10.3

Mean of 5 Male rats = 129.9 m.m. Hg. S.D. = 7.2 m.m. n = 25

Mean of 5 Female rats = 121.0 m.m. Hg. S.D. = 11.2 m.m. n = 25.

Weights of Rats (Grams.)

X1	-	335	
X2	-	335	
X3	-	330	Mean = 331.
X4	-	350	
X5	-	305	
Y1	-	240	
Y2	-	255	
Y3	-	245	Mean = 239.
Y4	-	225	
Y5	-	230	

Arterial Blood Pressures in m.m. Hg. after Injection of Guanethidine, 10 mgm./kg. at 10 a.m. (after initial B.P. measurement).

	10 a.m.	1 p.m.	4 p.m.	7 p.m.	10 p.m.	1 a.m.	4 a.m.	7 a.m.	10 a.m.
X1	132	104	124	110	108	104	128	98	136
X2	132	86	106	104	104	96	118	114	108
X3	120	106	108	110	106	110	118	114	112
X4	134	114	104	116	106	122	114	114	108
X5	120	122	118	106	112	116	110	124	110
Y1	124	96	106	90	94	88	110	106	106
Y2	130	108	88	92	104	112	124	100	136
Y3	126	90	92	104	110	102	122	106	118
Y4	122	86	110	86	94	88	90	96	92
Y5	120	94	88	88	86	88	92	104	114
Mean =	126.0	100.6	104.4	100.6	102.4	102.6	112.6	107.6	114.0
S.D. =	5.6	12.2	12.1	10.7	8.2	12.4	12.8	8.8	13.4

Pooled Means = 105.6 m.m. Hg.

Arterial Blood Pressures in m.m. Hg. After Injection of Hydralazine, 8 mgm./kg. at 10 a.m. (after initial B.P. measurement).

	10 a.m.	1 p.m.	4 p.m.	7 p.m.	10 p.m.	1 a.m.	4 a.m.	7 a.m.	10 a.m.
X1	140	84	86	108	96	120	108	124	112
X2	130	76	76	100	116	116	126	106	112
X3	120	76	84	96	108	112	96	102	118
X4	140	64	74	90	110	106	80	108	82
X5	128	60	80	104	108	124	102	104	110
Y1	124	64	90	104	96	88	82	100	96
Y2	126	78	78	106	114	120	106	112	116
Y3	130	76	86	92	100	112	94	106	100
Y4	128	66	86	96	96	110	92	76	104
Y5	112	58	60	88	106	96	106	108	98
Mean =	127.8	70.2	80.0	98.4	10.50	11.04	99.2	104.6	104.8
S.D. =	8.4	8.8	8.7	7.0	7.5	11.2	13.5	12.0	11.0

Pooled Mean = 96.6 m.m. Hg.

APPENDIX 2,A.

EXPERIMENT II.

Capillary Pressures in m.m. Hg.

Rat A 1. Normotensive.

18	34
20	34
22	34
24	36
24	38
26	38
26	38
32	40
32	42
32	42
32	46
32	

n = 23. Mean = 32.3 S.D. = 7.5

After Hydralazine.

20	46	56
20	46	60
22	46	
26	46	
28	46	
36	46	
38	46	
40	48	
40	50	
42	50	
42	52	
44	52	
44	56	

n = 28. Mean = 42.4 S.D. = 8.7

Rat A 1. Normotensive.

18	26	36	48
18	26	36	
20	28	36	
22	28	38	
22	28	38	
22	30	40	
22	32	42	
24	32	42	
24	34	42	
26	36	42	

n = 31. Mean = 30.3 S.D. = 8.1

After Guanethidine.

20	32	40
22	32	42
24	32	42
26	32	42
26	32	44
28	34	44
30	36	44
30	36	46
30	36	48
30	38	48

n = 30. Mean = 36.1 S.D. = 4.2

Rat A 2.	Normotensive.
16	30
18	30
18	32
18	32
24	32
24	34
26	34
28	36
28	36
28	38
28	42
28	42
30	46

n = 26. Mean = 29.9 S.D. = 7.6

After Hydralazine.

28	40
32	40
32	40
34	40
34	42
36	46
36	46
38	46
38	48
38	52
40	

n = 21. Mean = 39.3 S.D. = 5.7

Rat A 2. Normotensive.

18	28	36
22	28	38
22	30	38
24	30	38
24	30	38
24	32	38
26	32	42
26	34	42
26	34	44
26	36	44
28	36	

n = 32. Mean = 31.7 S.D. = 6.9

After Guanethidine.

22	32	42
24	32	44
24	36	44
26	38	44
26	38	46
26	38	46
28	40	46
30	40	46
30	40	48
32	40	48

n = 30. Mean = 37.0 S.D. = 5.6

Rat A 3. Normotensive.

20	36	42
22	36	42
22	36	42
24	36	42
26	36	42
28	38	46
32	38	
32	40	
34	40	
34	40	

n = 26. Mean = 34.8 S.D. = 7.2

After Hydralazine.

22	34	38
24	34	38
24	36	40
24	36	40
26	36	40
26	36	42
26	36	48
30	36	48
30	38	52
32	38	60

n = 30. Mean = 35.7 S.D. = 8.8

Rat A 3.		Normotensive.
22	26	34
22	26	34
22	28	38
24	30	38
24	30	40
24	30	40
26	30	42
26	32	42
26	34	44
26	34	48

n = 30. Mean = 31.4 S.D. = 7.3

After Guanethidine.

24	36	44
24	36	46
26	36	46
28	38	46
30	40	48
32	42	48
32	44	48
32	44	50
32	44	50
36	44	50

n = 30. Mean = 39.3 S.D. = 8.1

Rat Bl.		Normotensive.
20	32	40
20	32	40
20	32	40
22	32	42
22	34	44
22	34	44
26	34	
28	36	
30	36	
30	40	

n = 26. Mean = 32.0 S.D. = 7.7

After Hydralazine.

20	38	42
20	38	42
28	40	42
30	40	44
30	40	44
32	42	44
34	42	46
34	42	46
36	42	48
36	42	50

n = 30. Mean = 38.5 S.D. = 7.4

Rat Bl. Normotensive.

18	28	38	46
20	30	38	
20	30	38	
20	32	40	
22	32	40	
22	32	40	
22	34	40	
24	34	40	
24	36	44	
26	36	46	

n = 31. Mean = 32.0 S.D. = 8.4

After Guanethidine.

24	34	42
26	34	42
26	34	44
28	34	46
28	34	46
28	36	50
30	36	52
30	38	
30	40	
32	40	

n = 27. Mean = 35.7 S.D. = 7.6

Rat B2. Normotensive.

16	26	34
18	28	34
18	28	36
22	28	36
22	30	36
24	32	38
24	32	38
26	34	40
26	34	44
26	34	

n = 29. Mean = 29.8 S.D. = 7.0

After Hydralazine.

18	38	44
20	38	44
26	38	48
26	38	48
26	40	52
30	40	52
32	40	54
32	40	
36	40	
36	42	

n = 27. Mean = 37.7 S.D. = 9.3.

Rat B2. Normotensive.

18	26	30
20	26	30
20	26	32
20	26	34
22	28	34
22	28	36
22	28	36
24	28	38
24	30	38
24	30	38

n = 30. Mean = 27.9 S.D. = 5.9

After Guanethidine.

22	32	40
24	32	40
26	32	40
26	34	42
28	34	42
28	34	44
28	36	44
28	36	48
30	38	48
32	38	52

n = 30. Mean = 35.2 S.D. = 7.5

Rat B3. Normotensive.

16	22	30
16	24	32
18	24	34
18	28	34
20	28	34
22	28	36
22	28	36
22	30	40
22	30	40
22	30	44

n = 30. Mean = 27.7 S.D. = 7.4

After Hydralazine.

20	30	42
22	30	42
24	30	44
24	32	44
24	34	44
26	38	44
28	38	46
28	40	46
28	40	54
28	42	

n = 30. Mean = 35.1 S.D. = 8.9

Rat B3. **Normotensive.**

18	26	32
20	26	32
22	28	34
22	28	34
22	28	36
22	28	36
22	30	38
24	30	38
24	32	38
26	32	46

n = 30. Mean = 29.1 S.D. = 6.6

After Guanethidine.

24	30	40
24	32	42
26	32	42
26	32	44
26	34	46
26	34	48
28	36	48
28	36	48
28	38	
30	40	

n = 28. Mean = 35.5 S.D. = 7.2

Rat B 4.		Normotensive.
18	28	34
20	30	34
20	30	34
22	32	34
24	32	36
24	32	36
24	32	38
26	32	38
26	32	42
26	32	44

n = 30. Mean = 30.4 S.D. = 6.4

After Hydralazine.

22	30	36
22	32	36
24	32	38
24	32	38
24	32	38
26	34	38
28	34	38
28	34	38
28	34	40
30	34	46

n = 30. Mean = 32.3 S.D. = 5.9

Rat B 4. Normotensive.

18	28	36
20		
22	28	36
24	28	36
24	28	38
24	30	40
24	30	40
24	30	40
26	30	
	32	44
26	32	46
26	34	46

n = 32. Mean = 30.9 S.D. = 7.5

After Guanethidine.

20	34	40
24	36	42
24		
26	36	42
26	36	42
28		
30	38	42
32	38	44
32	38	46
34	38	48
34	38	
34	40	

n = 30. Mean = 35.4 S.D. = 6.0

Rat Cl. Normotensive.

16	24	32
20	24	32
20	26	34
20	26	34
20	26	34
22	26	34
22	26	36
22	28	38
22	28	42
22	30	

n = 29. Mean = 27.1 S.D. = 6.4

After Hydralazine.

24	34	40
26	36	42
26	36	42
28	36	42
30	38	44
30	38	46
34	38	46
34	38	46
34	40	48
34	40	50

n = 30. Mean = 37.3 S.D. = 6.7

Rat Cl. **Normotensive.**

18	26	32	44
20	28	32	46
22	28	32	
24	28	34	
24	28	36	
24	28	36	
26	30	38	
26	30	40	
26	30	40	
26	32	44	

n = 32. Mean = 30.6 S.D. = 7.0

After Guanethidine.

24	32	40
24	34	42
24	34	42
26	36	44
28	36	44
28	36	46
30	36	46
30	38	48
32	38	50
32	38	

m = 30. Mean = 35.7 S.D. 7.3

Rat C2. Normotensive.

22	30	42
26	30	42
26	32	46
28	32	46
28	32	48
28	32	
28	32	
30	34	
30	38	
30	42	

n = 26. Mean = 33.3 S.D. = 6.9

After Hydralazine.

22	34	40
22	34	40
24	34	42
26	36	42
26	36	44
28	36	44
28	36	46
30	36	46
32	38	50
34	48	56

n = 30. Mean = 36.0 S.D. = 8.2

Rat C2. Normotensive.

18	28	30
22	28	32
24	28	34
24	28	36
24	28	36
26	28	38
26	30	40
26	30	40
26	30	42
28	30	46

n = 30. Mean = 30.2 S.D. = 6.3

After Guanethidine.

20	32	38
22	32	38
24	32	40
24	34	40
26	34	44
26	34	46
26	36	48
28	36	48
28	38	50
28	38	50

n = 30. Mean = 34.7 S.D. = 8.6

Rat C3. Normotensive.

16	28	38
18	28	38
18	30	38
20	30	38
22	32	40
22	32	40
24	34	42
24	34	44
26	36	44
26	36	46

n = 30. Mean = 31.5 S.D. = 8.6

After Hydralazine.

18	30	38	42
22	32	38	
24	32	38	
24	32	38	
26	34	38	
26	34	40	
26	34	40	
26	36	42	
28	36	42	
30	38	42	

n = 31. Mean = 33.1 S.D. = 6.7

Rat C3. Normotensive.

20	28	34
20	28	34
20	28	34
22	28	34
22	30	36
22	30	36
22	30	38
24	30	38
24	32	40
26	34	46

n = 30. Mean = 29.3 S.D. = 7.3

After Guanethidine.

24	32	42
24	34	42
26	34	42
26	34	44
28	36	46
28	36	46
28	38	46
30	38	48
30	38	48
30	40	48

n = 30. Mean = 36.3 S.D.= 7.8

Rat C 4.		Normotensive.
18	26	32
20	26	32
20	26	34
20	28	38
20	28	38
22	30	38
22	30	38
22	30	42
24	30	44
26	32	46

n = 30. Mean = 29.4 S.D. = 7.7

After Hydralazine.

24	38	48
30	38	48
30	40	48
32	40	52
34	42	52
34	42	52
36	42 44	52
36		
38	44	60
38	46	
38	46	

n = 30. Mean = 41.5 S.D. = 8.0

Rat C 4. Normotensive.

	20	26	32	46
2	20	26	32	46
	22	26	32	
	22	26	38	
	22	26	38	
	22	28	40	
	24	28	40	
	24	28	44	
	24	28	44	
	26	32	46	

n = 32. Mean = 30.6 S.D. = 8.4

After Guanethidine.

	20	34	44
	22	34	46
	24	34	46
	24	34	48
	26	36	48
	28	38	48
	30	38	48
	30	40	48 50
	32	40	52
	32	44	

n = 30. Mean = 37.3 S.D. = 9.3

APPENDIX 2,B.

Normotensive Series I.

Group Boundary x values	$x - \mu$	$X=\frac{x-\mu}{S.D.}$	P(x)	$\triangle P(x)$	$305 \times \triangle P(x)$	O	O-E	$\frac{(O-E)^2}{E}$
15	-15.6295	-2.0682	.0193	less than 17				
17	-13.	-1.8036	.0356	0.0356	10.85	6	-4.85	2.17
19	-11.	-1.5389	.0619	.0263	8.02	12	3.98	1.97
21	-9.	-1.2743	.1013	.0394	12.02	17	4.98	2.06
23	-7.	-1.0096	.1563	.0550	16.78	26	9.22	5.07
25	-5.	-0.7449	.2282	.0719	21.93	18	-3.93	0.70
27	-3.	-0.4803	.3155	.0873	26.63	25	-1.63	0.10
29	-1.	-0.2156	.4146	.0991	30.23	25	-5.23	0.90
31	0.3705	0.0490	.5195	.1049	31.99	23	- 8.99	2.53
33	2.	0.3137	.6231	.1036	31.60	37	5.40	0.92
35	4.	0.5783	.7185	.0954	29,10	30	0.90	0.03
37	6.	0.8430	.8004	.0819	24.98	20	-4.98	0.99
39	8.	1.1077	.8660	.0656	20.01	20	-0.01	0.00
41	10.	1.3723	.9150	.0490	14.94	13	-1.94	0.25
43	12.	1.6370	.9492	.0342	10.43	17	6.57	4.14
45	14.	1.9016	.9714	.0222	6.77	8	1.23	0.22
47	16.	2.1663	.9849	above 45 .0286	8,74	8	-0.72	0.13
49	18.	2.4310	.9925					
					305.00	305	-10.01	22.18

Mean = 30.6295

S.D. = 7.5569

n = 305

Degrees of Freedom = 16 - 3 = 13.

The 5% point for chi-square with13 degrees of freedom is 22.36.

Normotensive Series II.

Group Boundary x values	$x - \mu$	$\frac{x-\mu}{s}$ X	P(x)	$\triangle P(x)$	$305 \times \triangle P(x)$	O	O-E	$\frac{(O-E)^2}{E}$
Less than 17				.0331	11.25	0	-11.25	11.25
17	-13.3824	-1.8377	.0331	.0258	8.77	9	0.23	0.01
19	-11.3824	-1.5630	.0589	.0399	13.57	20	6.43	3.05
21	- 9.3824	-1.2884	.0988	.0566	19.24	30	10.76	6.02
23	- 7.3824	-1.0137	.1554	.0745	25.33	34	8.67	2.97
25	- 5.3824	-0.7391	.2299	.0912	31.01	37	5.99	1.16
27	- 3.3824	-0.4645	.3211	.1036	35.22	39	3.78	0.41
29	- 1.3824	-0.1898	.4247	.1091	37.09	32	-5.09	0.70
31	0.6176	0.0848	.5338	.1066	36.24	25	-11.24	3.49
33	2.6176	0.3594	.6404	.0966	32.84	21	-11.84	4.27
35	4.6176	0.6341	.7370	.0812	27.61	22	5-5.61	1.14
37	6.6176	0.9087	.8182	.0635	21.59	25	3.41	0.54
39	8.6176	1.1834	.8817	.0459	15.61	18	2.39	0.37
41	10.6176	1.4580	.9276	.0308	10.47	9	-1.47	0.21
43	12.6176	1.7326	.9584	.0192	6.53	9	-2.47	0.93
45	14.6176	2.0073	.9776	.0224	7.62	10	2.38	0.74
above 45								
					339.99	340	+0.01	37.26

Mean = 30.3824
S.D. = 7.2823
n = 340.

Degrees of Freedom. There are 16 class intervals, therefore 15 degrees of freedom, but two other degrees must be subtracted as the sample mean and S.D. were used as an estimator of the population mean. The five per cent point for chi-square with 13 degrees of freedom is 22.36.

Total Normotensive.

Group Boundary x values	$x - \mu$	$X = \frac{x-\mu}{SD}$	P(x)	$\Delta P(x)$	$\Delta P(x) \times 645$	O	O-E	$\frac{(O-E)^2}{E}$
15	-15.4992	-2.0926	.0182	at less than 17 .0342	22.06	6	-16.06	11.69
17	-13.4992	-1.8226	.0342	.0261	16.83	21	4.17	1.03
19	-11.	-1.5525	.0603	.0395	25.48	37	11.52	5.21
21	-9.	-1.2825	.0998	.0559	36.06	56	19.94	11.03
23	-7.	-1.0125	.1557	.0732	47.21	52	4.79	0.49
25	-5.	-0.7425	.2289	.0894	57.66	62	4.34	0.33
27	-3.	-0.4724	.3183	.1015	65.47	64	-1.47	0.03
29	-1.	-0.2024	.4198	.1071	69.08	55	-14.08	2.87
31	+0.5008	0.0676	.5269	.1053	67.92	62	-5.92	0.52
33	+2.5008	0.3376	.6322	.0961	61.98	51	-10.98	1.94
35	+4.	0.6077	.7283	.0816	52.63	42	-10.63	2.15
37	+6.	0.8777	.8099	.0646	41.67	45	3.33	0.27
39	+8.	1.1477	.8745	.0474	30.57	31	0.43	0.01
41	+10.	1.4177	.9219	.0324	20.10	26	5.10	1.24
43	+12.	1.6878	.9543	.0206	13.29	17	3.71	1.04
45	+14.	1.9578	.9749	.0251	16.19	18	1.81	0.20
47	+16.	2.2278	more than 45		645.00	645	0	40.05
49	+18.	2.4978						

Mean = 30.4992 Degrees of Freedom = 14.

S.D. = 7.4067 The 5% point for chi-square with 14 degrees of freedom

n = 645 is 23.68.

After Hydralazine.

Group Boundary x values	$x - \mu$	X	P(x)	$\Delta P(x)$	$\Delta P(x)$ x317	O	O-E	$\frac{(O-E)^2}{E}$
17	-20.0662	-2.3762	.0087	less than 19				
19	-18	-2.1394	.0162	less than .0286	9.07	8	-1.07	0.13
21	-16	-1.9025	.0286	.0193	6.12	8	1.88	0.58
23	-14	-1.6657	.0479	.0286	9.07	14	4.93	2.68
25	-12	-1.4288	.0765	.0401	12.71	17	4.29	1.45
27	-10	-1.1920	.1166	.0531	16.83	14	-2.83	0.48
29	-8	-0.9552	.1697	.0666	21.11	17	-4.11	0.80
31	-6	-0.7183	.2363	.0788	24.98	16	-8.98	3.23
33	-4	-0.4815	.3151	.0883	27.99	26	-1.99	0.14
35	-2	-0.2446	.4034	.0935	29.64	27	-2.64	0.24
37	-0.0662	-0.0078	.4969	.0937	29.70	39	9.30	2.91
39	1.9338	0.2290	.5906	.0887	28.12	30	1.88	0.13
41	3	0.4658	.6793	.0795	25.20	29	3.80	0.57
43	5	0.7026	.7588	.0675	21.40	16	-5.40	1.36
45	7	0.9395	.8263	.0540	17.12	22	4.88	1.39
47	9	1.1763	.8803	.0409	12.97	11	-1.97	0.30
49	11	1.4132	.9212	.0293	9.29	5	-4.29	1.98
51	13	1.6500	.9505	.0199	6.31	10	3.69	2.16
53	15	1.8868	.9704	753.296	9.38	8	-1.38	0.20
55	17	2.1237	.9832					
57	19	2.3605	.9909					
59	21	2.5974	.9953					
61	23	2.8342	.9977					
					317.11	317	-0.01	21.73

Mean = 37.0662

S.D. = 8.4445

n = 317

Degrees of Freedom = 18 - 3 = 15

The 5% point for chi-square with 15 degrees of freedom is 25.00.

After Guanethidine.

Group Boundary x values	$x - \mu$	X	P(x)	$\Delta P(x)$	$\Delta P(x) \times 325$	O	O-E	$\frac{(O-E)^2}{E}$
19	-17.1969	-2.3254	.0100	less than 21				
				.0199	6.47	4	-2.47	1.56
21	-15	-2.0549	.0199					
				.0173	5.62	5	-0.62	0.07
23	-13	-1.7845	.0372					
				.0278	9.03	20	10.97	13.33
25	-11	-1.5140	.0650					
				.0418	13.59	23	9.41	6.52
27	-9	-1.2436	.1068					
				.0585	19.01	23	3.99	0.84
29	-7	-0.9731	.1653					
				.0758	24.63	21	-3.63	0.54
31	-5	-0.7027	.2411					
				.0917	29.80	30	0.20	0.00
33	-3	-0.4322	.3328					
				.1029	33.44	29	-4.44	0.59
35	-1	-0.1618	.4357					
				.1078	35.04	26	-9.04	2.33
37	0.8031	0.1085	.5435					
				.1042	33.86	26	-7.86	1.82
39	2.8031	0.3790	.6477					
				.0943	30.65	21	-9.65	3.04
41	4	0.6494	.7420					
				.0792	25.74	21	-4.74	0.87
43	6	0.9199	.8212					
				.0618	20.09	21	0.91	0.04
45	8	1.1903	.8830					
				.0450	14.62	21	6.38	2.78
47	10	1.4608	.9280					
				.0303	9.85	24	14.15	20.33
49	12	1.7312	.9583					
				.0190	6.18	8	1.82	0.54
51	14	2.0017	.9773					
				.0227	7.38	3	-4.38	2.60
53	16	2.2721	.9884					
					325.00	325	+0.38	60.93

Mean = 36.1969 Degrees of Freedom = 17 - 3 = 14.

S.D. = 7.3951 The 5% point for chi-square with 14 degrees of freedom

n = 325 is 23.68.

APPENDIX 2,C.

A1.

	1	2	3	4	5	6	7
	Normotensive Series 1.	After Hydralazine	Difference Col. 2-1	Normotensive Series II.	Difference Col. 4-1	After Guanethidine	Difference Col. 6-4
1	[illegible]	20		18		20	+2
2	18	20	+2	18	0	22	+4
3	20	22	+2	20	0	24	+4
4	22	26	+4	22	0	26	+4
5	24	28	+4	22	-2	26	+4
6	24	36	+12	22	-2	28	+6
7	26	38	+12	22	-4	30	+8
8	26	40	+14	24	-2	30	+6
9	32	40	+8	24	-8	30	+6
10				26		30	+4
		$\bar{x}$ = 7.25:s = 4.9		$\bar{x}$ = -2.25:s = 2.7		$\bar{x}$ = 4.8:s = 1.7	
1		42		26		32	+6
2	32	42	+10	26	-6	32	+6
3	32	44	+12	28	-4	32	+4
4	32	44	+12	28	-4	32	+4
5	32	46	+14	28	-4	32	+4
6	34	46	+12	30	-4	34	+4
7	34	46	+12	32	-2	36	+4
8	34	46	+12	32	-2	36	+4
9		46		34		36	+2
10		46		36		38	+2
		$\bar{x}$ = 12.0:s = 1.2		$\bar{x}$ =-3.7:s = 1.4		$\bar{x}$ = 4.0:s = 1.3	
1		46		36		40	+4
2	36	48	+12	36	0	42	+6
3	38	50	+12	36	-2	42	+6
4	38	50	+12	38	0	42	+4
5	38	52	+14	38	0	44	+6
6	40	52	+12	40	0	44	+4
7	42	56	+14	42	0	44	+2
8	42	56	+14	42	0	46	+4
9	46	60	+14	42	0	48	+6
10				48		48	0
		$\bar{x}$ = 13.05:s = 1.1		$\bar{x}$ = -.25:s = 0.7		$\bar{x}$ = 4.2:s = 2.0	

A2.

	1	2	3	4	5	6	7
1				22		22	
2	16			22	+6	24	+2
3	18	28	+10	24	+6	24	0
4	18	32	+14	24	+6	26	+2
5	18	32	+14	24	+6	26	+2
6	24	34	+10	26	+2	26	0
7	24	34	+10	26	+2	28	+2
8	26	36	+10	26	0	30	+4
9	28	36	+8	26	−2	30	+4
10	28			28	0	32	+4
		$\bar{x}$ = 10.9:s = 2.3		$\bar{x}$ = 2.9:s = 3.2		$\bar{x}$ = 2.2:s = 1.6	
1				28		32	+4
2	28	38	+10	28	0	32	+4
3	28	38	+10	30	+2	36	+6
4	28	38	+10	30	+2	38	+8
5	30	40	+10	30	0	38	+8
6	30	40	+10	32	+2	38	+6
7	30	40	+10	32	+2	40	+8
8	32	40	+8	34	+2	40	+6
9	32			34	+2	40	+6
10				36		40	+4
		$\bar{x}$ = 9.7:s = 0.7		$\bar{x}$ = 1.5:s = 0.9		$\bar{x}$ = 6.0:s = 1.6	
1				36		42	+6
2	32			36	+4	42	+6
3	34	40	+6	38	+4	44	+6
4	34	42	+8	38	+4	44	+6
5	36	46	+10	38	+2	46	+8
6	36	46	+10	38	+2	46	+8
7	38	46	+8	38	0	46	+8
8	42	48	+6	42	0	46	+4
9	42	52	+10	42	0	48	+6
10	46			44	−2	48	+4
		$\bar{x}$ = 8.3:s = 1.8		$\bar{x}$ = 1.6:s = 2.2		$\bar{x}$ = 6.2:s = 1.5	

A3.

	1	2	3	4	5	6	7
1		22		22		24	+2
2	20	24	+4	22	+2	24	+2
3	22	24	+2	22	0	26	+4
4	22	24	+2	24	+2	28	+4
5	24	26	+2	24	0	30	+6
6	26	26	0	24	-2	32	+8
7	28	26	-2	26	-2	32	+6
8	32	30	-2	26	-6	32	+6
9	32	30	-2	26	-6	34	+8
10	34	32	-2	26	-8	36	+10

$\bar{x}$ = 0.2:s = 2.4 $\bar{x}$ =-2.2:s = 3.7 $\bar{x}$ = 5.6:s = 2.6

1		34		26		36	+10
2	34	34	0	26	-8	36	+10
3	36	36	0	28	-8	36	+8
4	36	36	0	30	-6	38	+8
5	36	36	0	30	-6	40	+10
6	36	36	0	30	-6	42	+12
7	36	36	0	30	-6	44	+14
8	38	36	-2	32	-6	44	+12
9	38	38	0	34	-4	44	+10
10		38		34		44	+10

$\bar{x}$ = 0.5:s = 2.3 $\bar{x}$ =-6.25:s = 1.4 $\bar{x}$ = 10.4:s = 1.8

1		38		34		44	+10
2	40	38	-2	34	-6	46	+12
3	40	40	0	38	-2	46	+8
4	40	40	0	38	-2	46	+8
5	42	40	0	40	-2	48	+8
6	42	42	0	40	-2	48	+8
7	42	48	+6	42	0	48	+6
8	42	48	+6	42	0	50	+8
9	42	52	+10	44	+2	50	+6
10	46	60	+14	48	+2	50	+2

$\bar{x}$ = 3.7:s = 5.5 $\bar{x}$ =-1.1:s = 2.5 $\bar{x}$ = 7.6:s = 2.6

B1.

	1	2	3	4	5	6	7
	Normotensive Series 1.	After Hydralazine	Difference 1.	Normotensive Series II.	Difference 2.	After Guanethidine	Difference 3.
1		20		18			
2	20	20	0	20	0	24	+4
3	20	28	+8	20	0	26	+6
4	20	30	+10	20	0	26	+6
5	22	30	+8	22	0	28	+6
6	22	32	+10	22	0	28	+6
7	22	34	+12	22	0	28	+6
8	26	34	+8	24	-2	30	+6
9	28	36	+8	24	-4	30	+6
10	30	36	+6	26	-4	30	+4
		$\bar{x}$ = 7.8:s = 3.4		$\bar{x}$ = -1.1:s = 1.8		$\bar{x}$ = 5.5:s = 0.9	
1		38		28		32	+4
2	30	38	+8	30	0	34	+4
3	32	40	+8	32	0	34	+2
4	32	40	+8	32	0	34	+2
5	32	40	+8	32	0	34	+2
6	32	42	+10	34	+2	34	0
7	34	42	+8	34	0	36	+2
8	34	42	+8	36	+2	36	0
9	34	42	+8	36	+2	38	+2
10		42		38			
		$\bar{x}$ = 8.2:s = 0.7		$\bar{x}$ = .75:s = 1.0		$\bar{x}$ = 2.0:s = 1.4	
1		42		38		40	+2
2	36	42	+6	38	+2	40	+2
3	36	42	+6	40	+4	42	+2
4	40	44	+4	40	0	42	+2
5	40	44	+4	40	0	44	+4
6	40	44	+4	40	0	46	+6
7	40	46	+6	40	0	46	+6
8	42	46	+4	44	+2	50	+6
9	44	48	+4	46	+2	52	+6
10	44	50	+6	46	+2		
		$\bar{x}$ = 4.9:s = 1.1		$\bar{x}$ = 1.3:s = 1.4		$\bar{x}$ = 4.0:s = 2.0	

B2.

	1	2	3	4	5	6	7
1	16			18	+2	22	+4
2	18	18	0	20	+2	24	+4
3	18	20	+2	20	+2	26	+6
4	22	26	+4	20	-2	26	+6
5	22	26	+4	22	0	28	+6
6	24	26	+2	22	-2	28	+6
7	24	30	+6	22	-2	28	+6
8	26	32	+6	24	-2	28	+4
9	26	32	+6	24	-2	30	+6
10	26	36	+10	24	-2	32	+8
		$\bar{x}$ = 4.4:s = 2.9		$\bar{x}$ = -0.6:s = 1.9		$\bar{x}$ = 5.6:s = 1.2	
1				26		32	+6
2	26	36	+10	26	0	32	+6
3	28	38	+10	26	-2	32	+6
4	28	38	+10	26	-2	34	+8
5	28	38	+10	28	0	34	+6
6	30	38	+8	28	-2	34	+6
7	32	40	+8	28	-4	36	+8
8	32	40	+8	28	-4	36	+8
9	34	40	+6	30	-4	38	+8
10	34	40	+6	30	-4	38	+8
		$\bar{x}$ = 8.4:s = 1.7		$\bar{x}$ = -2.4:s = 1.7		$\bar{x}$ = 7.0:s = 1.1	
1	34			30	-4	40	+10
2	34	40	+6	30	-4	40	+10
3	34	42	+8	32	-2	40	+8
4	36	44	+8	34	-2	42	+8
5	36	44	+8	34	-2	42	+8
6	36	48	+12	36	0	44	+8
7	38	48	+10	36	-2	46	+10
8	38	52	+14	38	0	48	+10
9	40	52	+12	38	-2	48	+10
10	44	54	+10	38	-6	48	+10
		$\bar{x}$ = 8.8:s = 2.5		$\bar{x}$ = -2.4:s = 1.8		$\bar{x}$ = 9.2:s = 1.1	

B3.

	1	2	3	4	5	6	7
1	16	20	+4	18	+2	24	+6
2	16	22	+6	20	+4	24	+4
3	18	24	+6	22	+4	26	+4
4	18	24	+6	22	+4	26	+4
5	20	24	+4	22	+2	26	+4
6	22	26	+4	22	0	26	+4
7	22	28	+6	22	0	28	+6
8	22	28	+6	24	+2	28	+4
9	22	28	+6	24	+2	28	+4
10	22	28	+6	26	+4		
		$\bar{x}$ = 5.4:s = 0.9		$\bar{x}$ = 2.4:s = 1.5		$\bar{x}$ = 4.4:s = 0.9	
1	22	30	+8	26	+4	30	+4
2	24	30	+6	26	+2	30	+4
3	24	30	+6	28	+4	32	+4
4	28	32	+4	28	0	32	+4
5	28	34	+6	28	0	34	+6
6	28	38	+10	28	0	34	+6
7	28	38	+10	30	+2	34	+4
8	30	40	+10	30	0	36	+6
9	30	40	+10	32	+2	36	+4
10	30	42	+12	32	+2	38	+6
		$\bar{x}$ = 8.2:s = 2.6		$\bar{x}$ = +1.6:s = 1.5		$\bar{x}$ = 4.6:s = 0.9	
1	30	42	+12	32	+2		
2	32	43	+10	32	0	40	+8
3	34	42	+8	34	0	40	+6
4	34	44	+10	34	0	42	+8
5	34	44	+10	36	+2	42	+6
6	36	44	+8	36	0	44	+8
7	36	44	+8	38	+2	46	+8
8	40	46	+6	38	-2	48	+10
9	40	46	+6	38	-2	48	+10
10	44	54	+10	46	+2	48	+2
		$\bar{x}$ = 8.8:s = 1.9		$\bar{x}$ = +0.4:s = 1.5		$\bar{x}$ =7.3:s = 2.4	

B4.

	1	2	3	4	5	6	7
1	18	22	+4	18	0	20	+2
2	20	22	+2	20	0	24	+4
3	20	24	+4	22	+2	24	+2
4	22	24	+2	24	+2	26	+2
5	24	24	0	24	0	26	+2
6	24	26	+2	24	0	28	+4
7	24	28	+4	24	0	30	+6
8	26	28	+2	24	-2	32	+8
9	26	28	+2	26	0	32	+6
10	26	30	+4	26	0	34	+8
		$\bar{x}$ = 2.6:s = 1.3		$\bar{x}$ = +0.2:s = 1.1		$\bar{x}$ = 4.4:s = 2.5	
1	28	30	+2	28	0	34	+6
2	30	32	+2	28	-2	34	+6
3	30	32	+2	28	-2	34	+6
4	32	32	0	28	-4	36	+8
5	32	32	0	30	-2	36	+6
6	32	34	+2	30	-2	36	+6
7	32	34	+2	30	-2	38	+8
8	32	34	+2	30	-2	38	+8
9	32	34	+2	32	0	38	+6
10	32	34	+2	32	0	38	+6
		$\bar{x}$ = 1.6:s = 0.8		$\bar{x}$ = -1.6:s = 1.3		$\bar{x}$ =6.6:s = 0.9	
1	34	36	+2	34	0	38	+4
2	34	36	+2	36	+2	40	+4
3	34	38	+4	36	+2	40	+4
4	34	38	+4	38	+4	42	+4
5	36	38	+2	40	+4	42	+2
6	36	38	+2	40	+4	42	+2
7	38	38	0	40	+2	42	+2
8	38	38	0	44	+6	44	0
9	42	40	-2	46	+4	46	0
10	44	46	+2	46	+2	48	+2
		$\bar{x}$ = 1.6:s = 1.8		$\bar{x}$ = 3.0:s = 1.7		$\bar{x}$ = 2.4:s = 1.5	

Cl.

	1	2	3	4	5	6	7
	Normotensive Series 1.	After Hydralazine	Difference 1.	Normotensive Series 11.	Difference 2	After Guanethidine	Difference 3
1	16	24	+8	18	+2	24	+6
2	20	26	+6	20	0	24	+4
3	20	26	+6	22	+2	24	+2
4	20	28	+8	24	+4	26	+2
5	20	30	+10	24	+4	28	+4
6	22	30	+8	24	+2	28	+4
7	22	34	+12	26	+4	30	+4
8	22	34	+12	26	+4	30	+4
9	22	34	+12	26	+4	32	+6
10	22	34	+12	26	+4	32	+6
		$\bar{x}$ = 9.4:s =2.5		$\bar{x}$ = 3.0:s = 1.4		$\bar{x}$ = 4.2:s = 1.5	
1	24	34	+10	26	+2	32	+6
2	24	36	+12	28	+4	34	+6
3	26	36	+10	28	+2	34	+6
4	26	36	+10	28	+2	34	+6
5	26	38	+12	28	+2	36	+8
6	26	38	+12	30	+4	36	+6
7	26	38	+12	30	+4	36	+6
8	28	38	+10	30	+2	36	+6
9	28	40	+12	32	+4	38	+6
10		40		32		38	+6
		$\bar{x}$ = 11.1:s = 1.1		$\bar{x}$ = 2.9:s = 1.1		$\bar{x}$ = 6.2:s = 0.7	
1	30	40	+10	32	+2	38	+6
2	32	42	+10	32	0	40	+8
3	32	42	+10	34	+2	42	+8
4	34	42	+8	36	+2	42	+6
5	34	44	+10	38	+4	44	+6
6	34	46	+12	40	+6	44	+4
7	34	46	+12	40	+6	46	+6
8	36	46	+10	44	+8	46	+2
9	38	48	+10	44	+6	48	+4
10	42	50	+8	46	+4	50	+4
		$\bar{x}$ = 10.0:s = 1.3		$\bar{x}$ = 4.0:s = 2.5		$\bar{x}$ = 5.4:s = 19	

C2.

	1	2	3	4	5	6	7
1	22	22	0	18	-4	20	+2
2	26	22	-4	22	-4	22	0
3	26	24	-2	24	-2	24	0
4	28	26	-2	24	-4	24	0
5	28	26	-2	24	-4	26	+2
6	28	28	0	26	-2	26	0
7	28	28	0	26	-2	26	0
8	30	30	0	26	-4	26	0
9	30	32	+2	26	-4	28	+2
10		34		28		28	0
		$\bar{x}$ = -0.9:s = 1.5		$\bar{x}$ = -3.3:s = 1.0		$\bar{x}$ = 0.6:s = 0.9	
1		34		28		32	+4
2	30	34	+4	28	-2	32	+4
3	30	34	+4	28	-2	32	+4
4	30	36	+6	28	-2	34	+6
5	32	36	+4	28	-4	34	+6
6	32	36	+4	28	-4	34	+6
7	32	36	+4	30	-2	36	+6
8	32	36	+4	30	-2	36	+6
9	32	38	+6	30	-2	38	+8
10		38		30		38	+8
		$\bar{x}$ = 4.5:s = 0.9		$\bar{x}$ = -2.5:s = 0.9		$\bar{x}$ = 5.8:s = 1.5	
1		40		30		38	+8
2	32	40	+8	32	0	38	+6
3	34	42	+8	34	0	40	+6
4	38	42	+4	36	-2	40	+4
5	42	44	+2	36	-6	44	+8
6	42	44	+2	38	-4	46	+8
7	42	46	+4	40	-2	48	+8
8	46	46	0	40	-6	48	+8
9	46	50	+4	42	-4	50	+8
10	48	56	+8	46	-2	50	+4
		$\bar{x}$ = 4.4:s 2.0		$\bar{x}$ = -2.9:s = 2.2		$\bar{x}$ = 6.8:s = 1.7	

C3.

	1	2	3	4	5	6	7
1	16	18	+2	20	+4	24	+4
2	18	22	+4	20	+2	24	+4
3	18	24	+6	20	+2	26	+6
4	20	24	+4	22	+2	26	+4
5	22	26	+4	22	0	28	+6
6	22	26	+4	22	0	28	+6
7	24	26	+2	22	-2	28	+6
8	24	26	+2	24	0	30	+6
9	26	28	+2	24	-2	30	+6
10	26	30	+4	26	0	30	+4
		$\bar{x}$ = 3.4:s = 1.3		$\bar{x}$ = +0.6:s = 1.9		$\bar{x}$ = 5.2:s = 1.1	
1	28	30	+2	28	0	32	+4
2	28	32	+4	28	0	34	+6
3	30	32	+2	28	-2	34	+6
4	30	32	+2	28	-2	34	+6
5	32	34	+2	30	-2	36	+6
6	32	34	+2	30	-2	36	+6
7	34	36	+2	30	-4	38	+8
8	34	36	+2	30	-4	38	+8
9	36	38	+2	32	-4	38	+6
10	36	38	+2	34	-2	40	+6
		$\bar{x}$ = 2.2:s = 0.7		$\bar{x}$ = -2.2:s = 1.5		$\bar{x}$ = 6.2:s = 1.1	
1	38	38	0	34	-4	42	+8
2	38	38	0	34	-4	42	+8
3	38	38	0	34	-4	42	+8
4	38	38	0	34	-4	44	+10
5	40	40	0	36	-4	46	+10
6	40	40	0	36	-4	46	+10
7	42	42	0	38	-4	46	+8
8	44	42	-2	38	-6	48	+10
9	44	42	-2	40	-4	48	+8
10	46	42	-4	46	0	48	+2
		$\bar{x}$ = -0.8:s = 1.4		$\bar{x}$ = -3.8:s = 1.5		$\bar{x}$ = 8.2:s = 2.4	

C4.

	1	2	3	4	5	6	7
1	18	24	+6	20	+2	20	0
2	20	30	+10	20	0	22	+2
3	20	30	+10	22	+2	24	+2
4	20	32	+12	22	+2	24	+2
5	20	34	+14	22	+2	26	+4
6	22	34	+12	22	0	28	+6
7	22	36	+14	24	+2	30	+6
8	22	36	+14	24	+2	30	+6
9	24	38	+14	24	0	32	+8
10	26	38	+12	26	0	32	+6
		$\bar{x}$ = 11.8:s = 2.6		$\bar{x}$ = 1.2:s = 1.1		$\bar{x}$ = 4.2:s = 2.6	
1	26	38	+12	26	0	34	+8
2	26	38	+12	26	0	34	+8
3	26	38	+12	26	0	34	+8
4	28	40	+12	26	-2	34	+8
5	28	40	+12	26	-2	36	+10
6	30	42	+12	28	-2	38	+10
7	30	42	+12	28	-2	38	+10
8	30	42	+12	28	-2	40	+12
9	30	44	+14	28	-2	40	+12
10	32	44	+12	32	0	44	+12
		$\bar{x}$ = 12.2:s = 0.7		$\bar{x}$ = -1.2:s = 1.1		$\bar{x}$ = 9.8:s = 1.8	
1	32	46	+14	32	0	44	+12
2	32	46	+14	32	0	46	+14
3	34	48	+14	38	+4	46	+8
4	38	48	+10	40	+2	48	+8
5	38	48	+10	40	+2	48	+8
6	38	52	+14	44	+6	48	+4
7	38	52	+14	44	+6	48	+4
8	42	52	+10	46	+4	48	+2
9	44	52	+8	46	+2	50	+4
10	46	60	+14	46	0	52	+6
		$\bar{x}$ = 12.2:s = 2.4		$\bar{x}$ = 2.6:s = 2.3		$\bar{x}$ = 7.0:s = 3.8	

APPENDIX 2,D.

Variance Analysis Between the Two Normotensive Series.

Animals	Blocks 1 - 10	11 - 20	21 - 30	Animal Totals	Animal Means
1	-1.15	-1.85	-0.15	-3.15	-1.05
2	+1.45	0.75	0.8	+3.0	+1.00
3	-1.1	-3.15	-0.55	-4.8	-1.60
4	-0.55	0.4	0.65	+0.5	+0.17
5	-0.3	-1.2	-1.2	-2.7	-0.90
6	1.2	0.8	0.2	+2.2	+0.73
7	0.1	-0.8	1.5	+0.8	+0.27
8	1.5	1.45	2.0	+4.95	+1.65
9	-1.65	-1.25	-1.45	-4.35	-1.45
10	0.3	-1.1	-1.9	-2.7	-0.90
11	0.6	-0.6	1.3	+1.3	+0.43
Block Totals:	+0.4	-6.55	+1.2	-4.95	$\bar{x}$ = -0.15

Analysis of Variance.

Total Sum of Squares about the Mean = $(-1.15)^2 + (-1.85)^2 + \ldots + (1.3)^2 - \frac{(-4.95)^2}{33}$

= 50.4425 - 0.7424 = 49.7001.

Sum of Squares for Animals = $\frac{1}{3}\left\{(-3.15)^2 + (3.0)^2 + \ldots + (1.3)^2\right\} - \frac{(-4.95)^2}{33}$

= 35.7958 - 0.7424 = 35.0534.

Sum of Squares for Blocks = $\frac{1}{11}\left\{(+0.4)^2 + (-6.55)^2 + (+1.2)^2\right\} - \frac{(-4.95)^2}{33}$

= 4.0457 - 0.7424 = 3.3033.

Sources of Variation	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio
Animals	35.0534	10	3.5053	6.18
Blocks	3.3033	2	1.6516	2.91
Residual	11.3434	20	0.5672	
Total:	49.7001	32		

The 5% point of variance ratio distribution where $f_1 = 10$, $f_2 = 20$ is 2.35, when $f_1 = 2$, $f_2 = 20$ the point is 3.49. Therefore in this case there is a significant variation between animals but not between blocks.

Variance Analysis Between 1st. Normotensive Series and Series After Hydralazine.

Animals	Blocks 1 - 10	11 - 20	21 - 30	Animal Totals	Animal Means
1	3.65	6.0	6.5	16.15	5.38
2	5.45	4.85	4.15	14.45	4.82
3	0.1	0.25	1.85	2.20	0.73
4	3.9	4.1	2.45	10.45	3.48
5	2.2	4.2	4.4	10.80	3.93
6	2.7	4.1	4.4	11.20	3.73
7	1.3	0.8	0.8	2.90	0.97
8	4.7	5.55	5.0	15.25	5.08
9	-0.45	2.25	2.2	4.00	1.33
10	1.7	1.1	-0.4	2.40	0.80
11	5.9	6.1	6.1	18.10	6.03
Block Totals:	31.15	39.30	37.45	107.9	$\bar{x} = 3.27$

Analysis of Variance.

Sum of Squares about the Mean $= (3.65)^2 + (6.0)^2 + \ldots\ldots + (6.1)^2 - \frac{107.9}{33}$

$= 493.2950 - 352.8000 = \underline{140.495.}$

Sum of Squares for Animals $= \frac{1}{3}\left\{(16.15)^2 + (14.45)^2 + \ldots\ldots + (18.1)^2\right\} - \frac{107.9}{33}$

$= 472.0300 - 352.8000 = \underline{119.23.}$

Sum of Squares for Blocks $= {}^1/11\left\{(31.15)^2 + (39.30)^2 + (37.45)^2\right\} - \frac{107.9}{33}$

$= 356.1195 - 352.8000 = \underline{3.3195.}$

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	Variance Ratio
Animals	119.2300	10	11.9230	13.29
Blocks	3.3195	2	1.6597	1.85
Residual	17.9465	20	0.8973	
Total:	140.4950	32		

The 5% point of variance ratio distribution for $f_1 = 10$, $f_2 = 20$ is 2.35, for $f_1 = 2$, $f_2 = 20$ the point is 3.49. Therefore in this case, there is a significant variation between animals but not between the blocks.

Variance Analysis Between 2nd. Normotensive Series and Series After Guanethidine.

Animals	Blocks 1 - 10	11 - 20	21 - 30	Animal Totals	Animal Means
1	2.4	2.0	2.1	6.50	2.17
2	1.1	3.0	3.1	7.20	2.40
3	2.8	5.2	3.8	11.80	3.93
4	2.75	1.0	2.0	5.75	1.92
5	2.8	3.5	4.6	10.90	3.63
6	2.2	2.3	3.65	8.15	2.72
7	2.2	3.3	1.2	6.70	2.23
8	2.1	3.1	2.7	7.90	2.63
9	0.3	2.9	3.4	6.60	2.20
10	2.6	3.1	4.1	9.80	3.27
11	2.1	4.9	3.5	10.50	3.83
Block Totals:	23.35	34.30	34.15	91.80	$\bar{x}$ =2.78

Analysis of Variance.

Sum of Squares about the Mean = $(2.4)^2 + (2.0)^2 + ... + (3.5)^2 - \frac{(91.8)^2}{33}$

= 292.9650 - 255.3709 = 37.5941.

Sum of Squares for Animals = $\frac{1}{3}\left\{(6.5)^2 + (7.2)^2 + ... + (10.5)^2\right\} - \frac{(91.8)^2}{33}$

= 269.5917 - 255.3709 = 14.2208.

Sum of Squares for Blocks = $\frac{1}{11}\left\{(23.35)^2 + (34.30)^2 + (34.15)^2\right\} - \frac{(91.8)^2}{33}$

= 262.5395 - 255.3709 = 7.1686.

Sources of Variation	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio
Animals	14.2208	10	1.4221	1.76
Blocks	7.1686	2	3.5843	4.42
Residual	16.2047	20	0.8102	
Total:	37.5941	32		

The 5% point of variance ratio distribution for $f_1 = 10$, $f_2 = 20$ is 2.35, where $f_1 = 2$, $f_2 = 20$, the point is 3.49. Therefore, in this case, there is no significant variation between animals, but there is a significant variation between the blocks.

APPENDIX 3.A.

Experiment III.

	Animal.										
Day	A1	A2	A3	A4	B1	B2	B3	B4	B5	B6	Means.
2	2.4	2.0	1.8	1.3	2.4	2.0	2.6	2.5	2.3	2.9	2.22
4	2.2	2.0	1.7	2.7	2.7	2.2	2.4	1.8	2.1	2.3	2.21
6	1.3	1.6	1.8	2.0	1.3	1.3	1.0	2.1	0.9	1.2	1.45
8	2.2	1.2	2.0	1.0	1.9	1.3	1.0	2.1	0.9	1.2	1.63
10	1.5	2.1	1.7	2.0	2.0	1.9	1.6	1.2	2.3	1.7	1.80
12	1.8	1.6	1.6	2.0	1.4	2.0	1.2	1.9		2.1	1.73
14	1.8	1.5	1.5	1.3	1.2	1.1	1.8	1.3	1.2	1.3	1.40
16	1.4	1.9	2.3	1.6	1.8	1.9	2.0	1.7	1.9	2.4	1.89
18	1.6	2.1		2.0	1.1	1.6	1.3	1.3	1.1	1.4	1.50
20	1.5	1.3	1.7	1.9	1.4	1.4	1.1	2.3	1.6	1.1	1.53
22	1.9	2.1	2.0	1.6	1.5	2.0	2.3	1.4	1.6	1.9	1.83
24	D	1.3	1.3	1.4	1.5	1.9	1.8	1.4	1.6	1.0	1.47
26		2.0	1.7	B	1.4	1.7	2.4	1.7	1.9	1.9	1.84
28		1.3	1.7	0.8	D	1.3	1.4	1.7	1.7	2.1	1.50
30		1.6	1.6	1.0		1.7	2.4	1.0	1.5	2.2	1.63
32		2.1	1.8	1.9		2.0	1.3	1.7	1.8	2.0	1.82

Eruption Increments in Millimetres per 48 hours.

Group A (Control Group) Cut Incisors.

	Animal.											
Day	C1	C2	C3	C4	C5	D1	D2	D3	D4	D5	D6	Means.
2	2.5	1.9	1.6	1.8	B	2.2	2.8	1.2	1.7	2.4		2.01
4	2.2	1.9	2.3	1.9	2.1	2.1	1.9	2.1	2.5	2.2	2.7	2.17
6	1.9	1.5	1.6	1.3	1.3	2.0	1.0	1.6	0.9	1.2	2.4	1.52
8	1.6	2.4	2.3	1.8	1.7	1.0	1.7	1.2	1.0	2.2	1.3	1.65
10	2.1	1.6	2.0	1.8	1.6	1.8	1.4	1.8	1.2	1.7	1.7	1.70
12	1.8	1.2	2.2	2.0	1.8	1.9	1.6		1.7	1.4	2.1	1.77
14	2.0		1.7	1.7		1.4	1.7	1.8	2.1	1.8	1.7	1.77
16	1.5	1.9	1.9	1.7	1.9	2.2	1.7	1.6	1.5	1.9	1.6	1.77
18	2.1	2.4	1.6	D	1.7	1.4	1.7	1.1	1.5	1.9	2.1	1.75
20	1.7	1.3	2.0		2.2	2.1	1.2	1.7	2.1	1.5	1.3	1.71
22	1.7	2.2	1.8		1.4	1.8	1.4	1.8	1.4	2.0	1.7	1.72
24	2.0	1.3	1.8		1.9	1.3	1.7	1.6	1.4	2.2	B	1.69
26	1.1	2.1	1.2		1.9	1.5	2.1	2.1	1.7	2.1	0.7	1.65
28	1.5	1.8	2.1		2.2	2.1	1.5	2.4	1.6	1.8	1.7	1.87
30	1.9	1.9	2.0		1.1	1.1	1.9	0.8	2.1	1.1	1.3	1.52
32	1.7	1.8	1.6		1.5	1.3	1.9	1.9	2.2	2.1	1.8	1.78

Eruption Increments in Millimetres per 48 hours.

Group B (Guanethidine) Cut Incisors.

	Animal.										
Day	E1	E2	E3	E4	F1	F2	F3	F4	F5	F6	Means.
2	2.8	3.1	1.9	2.5	B	2.0	2.7	2.3	2.7	2.0	2.44
4	2.4	2.4	1.8	2.2	1.0	2.3	2.4	2.1	1.2	2.0	1.98
6	1.7	1.5	1.5	1.9	1.4	2.4	0.9	1.9	2.7	1.5	1.74
8	1.2	2.0	2.4	1.5	1.9	1.7	1.6	1.4	1.2	1.4	1.63
10	1.5	2.3	1.0	1.7	1.7	1.8	1.9	1.9	1.9	1.5	1.72
12	1.6	1.4	1.6	1.5	1.2	1.1	1.3	1.5	1.8	2.0	1.50
14	1.4	1.8	1.3	2.0	1.1	1.9	1.1	1.8	1.9	1.5	1.58
16	1.2	1.5	1.5	2.2	1.8	2.0	2.0	1.8	1.6	2.4	1.80
18	1.8	1.9	1.5	1.4	1.9	1.4	1.1	1.5	1.6	1.6	1.57
20	1.9	2.1	1.9	2.0	1.5	1.9	1.9	1.5	2.2	1.1	1.80
22	2.2	1.5	1.6	2.0	1.5	1.9	2.1	2.0	1.5	D	1.81
24	1.8	1.8	1.6	1.6	1.8	2.4	1.3	2.1	1.9		1.81
26	1.9	1.7	2.1	2.2	2.0	1.1	1.9	1.3	2.1		1.81
28	1.3	1.5	2.0	1.8	1.1	1.7	2.0	1.7	2.3		1.71
30	2.0	2.1	1.7	1.9	1.8	2.7	1.4	1.0	1.8		1.82
32	1.6	1.9	2.0	1.5	1.3	2.2	1.6	1.8	1.7		1.73

Eruption Increments in Millimetres per 48 hours.
Group C (Hydralazine) Cut Incisors.

	Animal.										
Day	A1	A2	A3	A4	B1	B2	B3	B4	B5	B6	Means.
2	1.7	1.7	1.7	0.6	1.6	1.5	2.0	2.1	1.8	2.5	1.72
4	1.4	0.9	0.7	1.6	1.5	1.3	1.7	0.8	1.3	1.5	1.27
6	1.4	0.9	1.1	1.1	0.5	0.3	0.2	1.4	0.4	0.5	0.78
8	1.3	0.3	1.1	0.5	0.6	0.4	0.9	0.5	0.4	1.2	0.72
10	0.8	1.2	1.0	1.1	1.3	1.1	1.0	0.3	1.9	1.1	1.08
12	0.6	1.0	0.6	1.0	0.6	0.8	0.5	1.1		1.2	0.82
14	1.2	0.6	0.5	0.7	0.3	0.4	0.7	0.5	0.4	0.5	0.58
16	0.7	0.7	1.2	0.9	1.0	0.4	1.2	0.8	1.0	1.5	0.94
18	0.9	1.4		1.2	0.2	0.8	0.7	0.4	0.4	0.6	0.73
20	0.7	0.3	1.0	0.8	0.5	0.6	0.1	1.4	0.9	0.4	0.67
22	1.3	1.3	1.6	0.7	1.2	0.9	1.3	0.8	0.9	1.6	1.16
24	D	0.8	0.5	0.3	0.7	1.0	0.8	0.8	0.9	0.1	0.66
26		0.8	1.1	B	0.8	1.5	1.3	0.9	0.8	1.1	1.04
28		0.2	0.8	0.3	D	0.4	0.7	0.9	0.5	1.1	0.61
30		0.7	0.3	0.1		0.8	1.5	0.2	0.7	0.5	0.60
32		0.9	0.4	0.3		0.5	1.0	1.2	1.0	1.3	0.83

Eruption Increments in Millimetres per 48 hours.
Group A (Controls) Uncut Incisors.

	Animal.											
Day	C1	C2	C3	C4	C5	D1	D2	D3	D4	D5	D6	Means.
2	1.7	1.6	1.3	1.6	B	1.5	2.2	0.9	1.4	1.9		1.57
4	0.8	0.8	1.5	1.0	1.4	0.7	1.4	1.5	1.6	1.7	2.1	1.32
6	1.1	1.3	0.5	0.8	0.8	1.2	0.3	0.7	0.3	0.8	1.6	0.85
8	0.5	1.2	0.9	1.0	1.2	0.3	1.1	1.2	0	1.7	0.5	0.87
10	1.3	0.8	1.0	1.2	1.0	1.0	0.7	0.9	0.1	1.2	1.1	0.94
12	1.0	0.6	1.3	0.8	1.0	0.9	0.8		0.7	0.8	0.9	0.88
14	0.9		0.7	1.0		1.1	0.9	1.2	1.3	0.8	0.8	0.97
16	0.8	1.1	1.2	1.1	1.2	1.4	0.9	0.7	1.1	1.2	1.0	1.06
18	1.2	1.5	0.9	D	1.2	0.6	0.9	0.3	0.5	1.5	1.4	1.00
20	0.6	0.4	1.2		1.3	1.1	0.8	1.1	0.4	0.6	0.7	0.82
22	0.8	1.0	1.0		0.5	1.2	0.8	1.0	1.2	1.2	0.5	0.92
24	1.5	0.3	0.8		1.2	0.5	1.4	0.9	0.6	0.5	B	0.86
26	0.1	1.1	0.7		1.3	0.6	1.1	1.1	0.9	0.5	0.2	0.76
28	0.8	0.9	1.0		1.5	1.3	0.5	1.1	0.5	1.1	0.7	0.94
30	0.9	1.0	0.7		0.2	0.2	1.0	0.2	1.4	0.7	0.1	0.64
32	0.5	1.2	0.7		1.2	0.9	1.4	1.2	1.0	0.3	0.8	0.92

Eruption Increments in Millimetres per 48 hours.
Group B (Guanethidine) Uncut Incisors.

	Animal.										
Day	E1	E2	E3	E4	F1	F2	F3	F4	F5	F6	Means.
2	2.0	2.5	1.9	1.9	B	1.5	1.9	1.9	1.6	1.8	1.89
4	1.9	1.4	1.2	1.4	0.5	1.9	1.3	1.3	0.7	1.4	1.30
6	1.0	0.7	0.9	0.8	0.3	1.8	0.6	1.5	1.8	0.5	0.99
8	0.7	1.3	1.5	0.8	1.4	0.2	0.9	1.2	0.3	0.6	0.89
10	1.0	1.4	0.8	1.2	0.7	1.0	1.2	1.3	1.1	1.0	1.07
12	1.0	0.3	0.8	1.0	0.4	0.2	0.8	0.5	1.2	1.3	0.75
14	0.8	0.7	0.6	0.8	0.3	1.0	0.4	1.4	1.1	0.1	0.72
16	0.6	0.4	0.8	1.6	0.8	0.8	0.9	1.1	1.0	1.5	0.95
18	1.1	1.2	0.9	0.6	1.2	0.8	0.3	0.8	0.8	0.6	0.83
20	0.9	1.5	1.2	1.2	0.5	0.7	0.9	0.9	1.4	0.2	0.94
22	1.4	0.4	0.8	1.0	0.5	1.5	1.5	1.5	0.5	D	1.01
24	1.0	1.0	0.9	0.6	1.1	1.1	0.5	1.1	1.1		0.93
26	0.7	1.2	1.1	1.5	1.2	0.3	0.9	0.2	0.7		0.87
28	0.7	0.6	1.0	1.0	0.2	0.5	1.2	0.8	1.7		0.86
30	1.1	1.0	0.8	1.1	1.0	1.9	0.1	0.2	0.2		0.82
32	0.6	1.1	0.5	0.4	0.8	1.2	0.8	1.0	0.7		0.79

Eruption Increments in Millimetres per 48 hours.
Group C (Hydralazine) Uncut Incisors.

APPENDIX 3,B.

Experiment III. **Weights in Gms.**

Group A.

Day	A1	A2	A3	A4	B1	B2	B3	B4	B5	B6
0	275	270	265	265	175	190	200	180	180	185
2		280	275	275	170	200	205	185	190	190
4		280	275	280	170	195	200	185	195	190
6	285		285		170	195	200	190	195	190
8	285	280		285	170	195	205	190	195	185
10	290	285	290	285	170	190	205	190	200	185
12	285	285	285		170	195	210	190	195	190
14	285	290	285	295	175	200	210	190	195	190
16	285	285	285	295	170	195		190		190
18	290	290		300	170	195	205	190	200	190
20	295	295	290	300		200	210	195	205	195
22	Died	300	285	305	180	200	210	190	200	195
24			290	307	175	205	210	195		195
26		295	290	305	180	200	205	195	205	190
28		295	290	310	Died	205	210	195	200	195
30		300	295	310		205	210	195	205	195
32		300	295	310		205	215	200	205	195

Weights in Gms.

Group B.

Day	C1	C2	C3	C4	C5	D1	D2	D3	D4	D5	D6
0	275	255	285	260	295	175	195	205	185	190	175
2	280	260	286	260	300	175	195	210		190	175
4	280	265	290	265	305	175	195	215	200	195	185
6	285		300	275	300	180	200	215	200	195	185
8	290	260	305	275	300	175	195	215	205	190	185
10			305	280	300	175	200	220		190	
12	290	265	300	275	305	175	195	215	195		185
14	290	270	300	280	305	180	200	215	195	195	
16	300	270	305	D	310	180	200	210	200	195	185
18	300	280	305		310	180	200	210	205	195	190
20	300	275	305		315	180	205	215	205	200	190
22	305	280	305			180	205	215	200	200	195
24	305	280	300		315	180	200	215	205	195	190
26	310	275	305		320	180	205	215	205	195	185
28	315	280	305		315	180	200	215	205	195	185
30	315	280	305		320	185	205	220		200	
32	315	285	305		320	185	205	215	205		185

Weights in Gms.

Group C.

Day	E1	E2	E3	E4	F1	F2	F3	F4	F5	F6
0	300	240	245	330	160	185	190	185	205	210
2	300	245	245	325	170	195	195	190	210	205
4	310	255	250	320	170	190	190	185	210	205
6		260	245	330	170	190	195	190	205	215
8	310	260	245		165	190	195	185	210	215
10	310	265	245	340	165	185	190	180	205	215
12	310	260	245	330	170	190	195	175	205	205
14	310	260	245	330	170	185	195	175		205
16	315	265	245	335	170	195	200	180	205	205
18	315	270	250	340	170	200	200	180	205	210
20	320		250	340	170	195	200	180	200	210
22	320	275		340	175	200	205	185	210	D
24	325	280	265	340	180	200	205	190	215	
26	330	275	265	350	175	200	200	185	215	
28	330	280	265	350	180	200	205	190	215	
30	330	280	270	350	180		205	190	215	
32	330	285	270	350	180	205	205	190	215	

APPENDIX 3,C.

Group Boundary x values	$x - \mu$	$X = \frac{(X-\mu)}{S}$	P(X)	$\Delta P(X)$	E = $\Delta P(X) \times 93$	O	O − E	$\frac{(O-E)^2}{E}$	E
0	-.9097	-2.1952	.0141						
				.0049	0.46	1			
0.05	-.8597	-2.0746	.0190						
				.0144	1.34	1			less than
0.15	-.7597	-1.8333	.0334						
				.0223	2.07	2	+2.58	0.54	0.45
0.25	-.6597	-1.5919	.0557						
				.0327	3.04	8			= 12.42
0.35	-.5597	-1.3506	.0884						
				.0452	4.20	3			
0.45	-.4597	-1.1093	.1336						
				.0591	5.50	7	1.50	0.41	
0.55	-.3597	-0.8680	.1927						
				.0727	6.76	3	-3.76	2.09	
0.65	-.2597	-0.6267	.2654						
				.0846	7.87	5	-2.87	1.05	
0.75	-.1597	-0.3854	.3500						
				.0927	8.62	8	-0.62	0.04	
0.85	-.0597	-0.1441	.4427						
				.0960	8.93	6	-2.93	0.96	
0.95	.0403	0.0972	.5387						
				.0938	8.72	10	1.28	0.19	
1.05	.1403	0.3386	.6325						
				.0865	8.04	10	1.96	0.48	
1.15	.2403	0.5799	.7190						
				.0752	6.99	11	4.01	2.30	
1.25	.3403	0.8212	.7942						
				.0618	5.75	6	0.25	0.01	
1.35	.4403	1.0625	.8560						
				.0479	4.45	4	-0.45	0.04	more than
1.45	.5403	1.3038	.9039						
				.0349	3.25	3			1.45
1.55	.6403	1.5451	.9388						
				.0242	2.25	1	-0.75	0.06	=8.75
1.65	.7403	1.7864	.9630						
				.0157	1.46	1			
1.75	.8403	2.0278	.9787						
				.0097	0.90	2			
1.85	.9403	2.2691	.9884						
				.0056	0.52	1			
1.95	1.0403	2.5104	.9940						
				.9799	92.80	93	+0.20	8.17	

Goodness of fit to a normal distribution test.

All Groups. Uncut Incisors day 6 - day 10.

Mean = 0.9097

S.D. = 0.4144

n = 93.

Degrees of Freedom = 12 - 3 = 9.

The 5% point for chi-square with 9 degrees of freedom is 16.92.

Group Boundary x values	x -	$X = \frac{(X -)}{S}$	P(X)	P(X)	E = 129X P(X)	0	0 - E	$\frac{(0-E)^2}{E}$	E
0	-.8047	-2.0777	.0189						
				.0064	0.83				
0.05	-0.7547	-1.9556	.0253						
				.0202	2.61	3	-2.80	0.80	less than
0.15	-0.6547	-1.6904	.0455						
				.0305	3.93	4			0.25 = 9.80
0.25	-0.5547	-1.4322	.0760						
				.0442	5.70	9	3.30	1.91	
0.35	-0.4547	-1.1740	.1202						
				.0597	7.70	11	3.30	1.41	
0.45	-0.3547	-0.9158	.1799						
				.0755	9.74	13	3.26	1.09	
0.55	-0.2547	-0.6576	.2554						
				.0894	11.53	7	-4.53	1.78	
0.65	-0.1547	-0.3994	.3448						
				.0991	12.78	11	-1.78	0.25	
0.75	-0.0547	-0.1412	.4439						
				.1027	13.25	14	0.75	0.04	
0.85	0.0453	0.1170	.5466						
				.0996	12.85	11	-1.85	0.27	
0.95	0.1453	0.3752	.6462						
				.0906	11.69	10	-1.69	0.24	
1.05	0.2453	0.6334	.7368						
				.0768	9.91	10	0.09	0.01	
1.15	0.3453	0.8915	.8136						
				.0613	7.91	9	1.09	0.15	
1.25	0.4453	1.1498	.8749						
				.0455	5.87	7	1.13	0.22	more than
1.35	0.5453	1.4080	.9204						
				.0318	4.10	4			1.35 = 10.27
1.45	0.6453	1.6662	.9522						
				.0206	2.66	3			
1.55	0.7453	1.9243	.9728						
				.0127	1.64	2	-0.27	0.02	
1.65	0.8453	2.1825	.9855						
				.0072	0.93				
1.75	0.9453	2.4407	.9927						
				.0038	0.49				
1.85	1.0453	2.6989	.9965						
				.0019	0.25	1			
1.95	1.1453	2.9571	.9984						
				0.9795	129.00	129	0	8.19	

Goodness of fit to a normal distribution test.

Group A (Controls) Uncut Incisors, day 6 - 32.

Mean = 0.8047

S.D. = 0.3873

n = 129.

Degrees of Freedom = 13 - 3 = 10.

The 5% point for chi-square with 10 degrees of freedom is 18.31.

Group Boundary x values	$x - \mu$	$X = \frac{(x-\mu)}{S}$	P(X)	$\triangle P(X)$	$E = 93X \triangle P(X)$	0	0 - E	$\frac{(0-E)^2}{E}$	E
0.85	-.7954	-2.0151	.0219	.0171	1.59	3			
0.95	-.6984	-1.7627	.0390	.0265	2.46	6	-0.69	0.05	
1.05	-.5984	-1.5103	.0655	.0387	3.60	0			less than
1.15	-.4984	-1.2580	.1042	.0531	4.94	8	3.06	1.90	1.15=9.69
1.25	-.3984	-1.0056	.1573	.0684	6.36	7	0.64	0.06	
1.35	-.2984	- .7532	.2257	.0826	7.68	4	-3.68	1.76	
1.45	-.1984	- .5008	.3083	.0936	8.70	8	-0.70	0.06	
1.55	-.0984	-.2484	.4019	.0997	9.27	10	0.73	0.05	
1.65	.0016	+ .0040	.5016	.0996	9.26	11	1.74	0.33	
1.75	.1016	.2564	.6012	.0934	8.69	7	-1.69	0.33	
1.85	.2016	.5088	.6946	.0821	7.64	9	1.36	0.24	
1.95	.3016	.7612	.7767	.0679	6.31	7	0.69	0.08	
2.05	.4016	1.0136	.8446	.0526	4.89	3	-1.89	0.73	more than
2.15	.5016	1.2660	.8972	.0384	3.57	2			2.15=9.56
2.25	.6016	1.5184	.9356	.0261	2.43	3	0.44	0.02	
2.35	.7016	1.7708	.9617	.0168	1.56	4			
2.45	.8016	2.0232	.9785			1			
				.9781	92.99	93	+0.01	5.61	

Goodness of Fit to a Normal Distribution Test.

All groups. Cut Incisors. Day 6 - Day 10.

Mean = 1.6484. Degrees of Freedom = 12 - 3 = 9.

S.D. = 0.3962. The five per cent point for chi-square with

n = 93. 9 d.f. is 16.92.

Group Boundary x values	$x - \mu$	$X = \frac{(X-\mu)}{\sigma}$	P(X)	$\Delta P(X)$	$E = 129X\Delta P(X)$	O	O - E	$\frac{(O-E)^2}{E}$	E
0.75	-0.8926	-2.4623	.0069	.0075	0.97	1			
0.85	-0.7926	-2.1865	.0144	.0136	1.75	1	0.42	0.03	less than 1.05
0.95	-0.6926	-1.9106	.0280	.0230	2.97	5			= 6.58
1.05	-0.5926	-1.6348	.0510	.0361	4.66	4	-0.66	0.09	
1.15	-0.4926	-1.3589	.0871	.0523	6.75	7	0.25	0.01	
1.25	-0.3926	-1.0830	.1394	.0704	9.08	15	5.92	3.86	
1.35	-0.2926	-0.8072	.2098	.0878	11.33	10	-1.33	0.16	
1.45	-0.1926	-0.5313	.2976	.1016	13.11	8	-5.11	1.99	
1.55	-0.0926	-0.2554	.3992	.1089	14.05	15	0.95	0.06	
1.65	0.0074	0.0204	.5081	.1084	13.98	13	-0.98	0.07	
1.75	0.1074	0.2963	.6165	.0999	12.89	9	-3.89	1.17	
1.85	0.2074	0.5721	.7164	.0854	11.02	13	1.98	0.36	
1.95	0.3074	0.8480	.8018	.0677	8.73	13	4.27	2.09	
2.05	0.4074	1.1239	.8695	.0497	6.41	6	-0.41	0.03	more than 2.15
2.15	0.5074	1.3997	.9192	.0339	4.37	2			= 10.42
2.25	0.6074	1.6756	.9531	.0214	2.76	4	-1.42	0.19	
2.35	0.7074	1.9514	.9745	.0125	1.61	3			
2.45	0.8074	2.2273	.9870						
				.9801	129.01	129	-0.01	10.11	

Goodness of Fit to a Normal Distribution Test.

Group A - Controls. Cut Incisors. Day 6 - 32.

Mean = 1.6426. Degrees of Freedom = 13 - 3 = 10.

S.D. = 0.3625. The 5% point for chi-square with 10 degrees of freedom

n = 129. is 18.31.

APPENDIX 4,A.

Animal		Day. 2	4	6	8	10	12	14	16	18	20	22
A	1	1.85	1.87	1.57	2.06	1.87	1.76	1.68	1.38	1.68	1.96	1.68
	2	2.24	2.34	1.78	2.15	1.98	1.60	1.87	1.67	2.24	1.59	1.79
	3	1.98	2.26	1.85	2.24	2.15	1.96	1.87	1.78	1.94	1.98	1.87
	4	1.96	2.15	1.96	1.67	1.78	1.76	1.40	1.76	1.39	1.87	1.70
B	1	1.87	1.98	1.87	1.96	2.15	1.87	1.68	1.48	2.06	1.87	1.79
	2	2.34	1.59	1.50	1.94	1.50	1.87	1.60	1.59	1.93	1.51	1.79
	3		1.60	1.87	2.17	1.70	1.79	1.70	1.70	1.96	1.98	1.98
	4	1.96	1.79	1.78	2.13	1.76	1.96	1.42	1.59	1.89	2.08	1.89
C	1	2.13	1.78	1.74	1.57	1.76	1.85		1.65	1.65	1.94	1.67
	2	1.96	2.26	1.68	2.08	1.96	1.85	1.78	1.60	1.40	1.96	1.67
	3	2.06	1.79	1.79	1.96	1.60	1.96	1.98	1.87	1.98	1.78	1.68
	4	2.08	2.17	1.78	2.04	1.87	1.94	2.26	1.67	2.41	1.96	1.59

Eruption Increments in Millimetres per 48 hours, corrected individually for radiographic distortion as described in the text.

Pooled mean = 1.8506 : standard deviation = 0.2175.

Group Boundary x values	$x - \mu$	$X = \frac{X-\mu}{S}$	P(X)	$\triangle P(X)$	$E = 130 \times \triangle P(X)$	O	O - E	$\frac{(O-E)^2}{E}$	E
1.35	-0.5006	-2.3016	.0107						
				.0220	2.86	5	-1.86	0.32	less than
1.45	-0.4006	-1.8418	.0327						
				.0508	6.60	4			1.55 = 10.86
1.55	-0.3006	-1.3821	.0835						
				.0947	12.31	12	-0.31	.01	
1.65	-0.2006	-0.9223	.1782						
				.1437	18.68	18	-0.68	..02	
1.75	-0.1006	-0.4625	.3219						
				.1770	23.01	19	-4.01	0.70	
1.85	-0.0006	-0.0028	.4989						
				.1773	23.05	26	2.95	0.38	
1.95	0.0994	0.4570	.6762						
				.1442	18.75	22	3.25	0.56	
2.05	0.1994	0.9168	.8204						
				.0953	12.39	10	-2.39	0.46	
2.15	0.2994	1.3766	.9157						
				.0511	6.64	8			more than
2.25	0.3994	1.8363	.9668						
				.0224	2.91	5	3.04	0.84	2.15 = 10.96
2.35	0.4994	2.2961	.9892						
				.0079	1.03	1			
2.45	0.5994	2.7559	.9971						
				0.9864	130.01	130	-0.01	3.29	

Mean = 1.8506
S.D. = 0.2175
n = 130.

Degrees of Freedom = 8 - 3 = 5.
The 5% point for chi-square with 5 degrees of freedom is 11.07.

Radiographic Test Group.

APPENDIX 4B.

Day	0	2	4	6	8	10	12	14	16	18	20	22
A1	117.3	97.7	18.0	97.2	100.5	100.5	99.7	100.5	98.8	102.2	102.2	100.5
2	120.5	94.7	98.1	98.1	97.2	103.3	105.0	103.3	97.2	99.8	102.4	103.3
3	126.8	106.9	106.0	99.6	101.4	99.6	95.1	97.8	94.2	97.8	99.6	97.8
4	104.8	97.6	101.6	104.8	104.0	96.8	100.0	100.0	96.8	100.0	96.8	104.8
B1	117.5	96.0	97.7	99.3	98.5	99.3	96.0	101.8	101.0	101.0	106.0	106.0
2	95.4	93.2	96.1	95.4	99.7	101.2	101.2	101.9	104.0	101.2	104.0	105.5
3		123.6	106.7	101.1	101.1	99.3	99.3	96.4	97.4	99.3	99.3	99.3
4	112.2	101.6	102.4	99.2	97.6	95.9	95.9	99.2	100.0	102.4	105.7	100.8
C1	115.6	95.5	97.1	100.2	100.2	97.1	99.4		100.2	104.8	104.0	104.8
2	115.1	97.3	99.4	99.4	99.4	99.4	100.9	100.9	101.6	103.0	99.4	99.4
3	126.5	101.5	103.2	101.5	99.0	98.2	98.2	99.8	99.8	96.5	101.5	101.5
4	118.8	96.8	99.7	98.2	99.0	101.2	96.8	99.7	101.2	99.7	105.6	105.6

Length of right incisor converted to percentage of mean length of each incisor for the period of the experiment, omitting the first measurement when calculating the mean.

Day.

Animal	0	2	4	6	8	10	12	14	16	18	20	22
A 1	70	59.60	58.59	58.58	60.60	60.60	60.59	60.60	59.59	61.61	61.61	60
2	70	55.55	57.57	57.57	56.57	60.60	61.61	60.60	56.57	58.58	59.60	60
3	70	59.59	59.58	55.55	56.56	55.55	52.53	54.54	52.	54.54	55.55	54
4	66	62.61	63.65	66.66	65.66	61.61	63.63	63.63	61.61	63.63	61.61	66
B 1	71	59.57	59.59	60.60	59.60	60.60	58.58	63.60	61.61	61.61	63.65	64
2	66	64.65	66.67	66.66	69.69	70.70	70.70	70.71	72.72	70.70	72.72	73
3		66	57.57	54.54	54.54	53.53	53.53	51.52	52.52	53.53	53.53	53
4	69	62.63	63.63	61.61	60.60	59.59	59.59	60.62	61.62	63.63	65.65	62
C 1	75	62.62	63.63	64.66	65.65	63.63	65.64		65.65	68.68	68.67	68
2	81	68.69	70.70	70.70	70.70	70.70	70.72	71.71	71.72	71.74	70.70	70
3	76	61.61	62.62	61.61	59.60	59.59	59.59	60.60	60.60	58.58	61.61	61
4	81	66.66	68.68	67.67	68.67	69.69	66.66	68.68	69.69		72.72	72

Lengths (uncorrected) in millimetres of the uncut incisor.
Duplicate measurements from day 2 to day 20.
When the differences between the duplicate measurements are analysed, the mean difference between first and second reading is +0.0164, standard deviation 0.075.

Day.

Animal	2	4	6	8	10	12	14	16	18	20	22	Animal Totals	Animal Mean
A 1	1.85	1.87	1.57	2.06	1.87	1.76	1.68	1.38	1.68	1.96	1.68	19.36	1.76
2	2.24	2.34	1.87	2.15	1.98	1.60	1.87	1.67	2.24	1.59	1.79	21.34	1.94
3	1.98	2.26	1.85	2.24	2.15	1.96	1.87	1.78	1.94	1.98	1.87	21.88	1.99
4	1.96	2.15	1.96	1.67	1.78	1.76	1.40	1.76	1.39	1.87	1.70	19.40	1.77
B 1	1.87	1.98	1.87	1.96	2.15	1.87	1.68	1.48	2.06	1.87	1.79	20.58	1.87
2	2.34	1.59	1.50	1.94	1.50	1.87	1.60	1.59	1.93	1.51	1.79	19.16	1.74
3	1.85	1.60	1.87	2.17	1.70	1.79	1.70	1.70	1.96	1.98	1.98	20.30	1.85
4	1.96	1.79	1.78	2.13	1.76	1.96	1.42	1.59	1.89	2.08	1.89	20.25	1.84
C 1	2.13	1.78	1.74	1.57	1.76	1.85	1.85	1.65	1.65	1.94	1.67	19.59	1.78
2	1.96	2.26	1.68	2.08	1.96	1.85	1.78	1.60	1.40	1.96	1.67	20.20	1.84
3	2.06	1.79	1.79	1.96	1.60	1.96	1.98	1.87	1.98	1.78	1.68	20.45	1.86
4	2.08	2.17	1.78	2.04	1.87	1.94	2.26	1.67	2.41	1.96	1.59	21.77	1.98
Day Totals:	24.28	23.58	21.26	23.97	22.08	22.17	21.09	19.74	22.53	22.48	21.10	G= 244.28	$\bar{x}$ = 1.85
Day Mean:	2.02	1.97	1.77	2.00	1.84	1.85	1.76	1.65	1.88	1.87	1.76		

Correction Factor, $C = G^2/b+ = 452.0660$

Total sum of squares about the mean = $(1.85)^2 + (1.87)^2 + ... + (1.59)^2 - C =$
$458.1664 - 452.0660 = 6.1004.$

Sum of squares for animals = $^1/_{11} (19.36)^2 + (21.34)^2 + ... + (21.77^2) - C =$
$452.9125 - 452.0660 = 0.8465.$

Sum of squares for days = $^1/_{12} (24.28^2) + (23.58)^2 + ... + (21.10)^2 - C =$
$453.6446 - 452.0660 = 1.5786.$

Analysis of Variance.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio
Animals	0.8465	11	0.0770	2.31
Days	1.5786	10	0.1579	4.73
Residual	3.6753	110	0.0334	
Total:	6.1004	131	-	

The variation between animals is not significant, the 5% point of variance ratio distribution with $f_1 = 110$, $f_2 = 11$ is 2.45.

Variation between days is significant, the 5% point of variance ratio distribution with $f_1 = 110$, $f_2 = 10$ is 2.59, the 1% point is 4.01.

APPENDIX 6,A.

Day	CM1	CM2	CM3	CF1	CF2	CF3	Mean.
2	1.88	1.96	1.96	1.79	1.96	1.70	1.87
4	1.96	2.14	1.96	1.79	2.14	1.88	1.98
6	1.70	1.96	1.88	1.70	1.96	1.88	1.85
8	1.88	2.32	1.79	1.70	1.96	1.70	1.89
10	2.05	2.14	1.79	1.88	1.88	1.79	1.92
12	1.70	1.88	1.79	1.79	1.96	1.70	1.80
14	1.88	2.05	2.05	1.79	1.88	1.70	1.89
16	1.88		1.70	1.79	2.05	2.14	1.91
18	2.32		2.05	1.88	1.88	2.14	2.05
20	2.14		2.14	1.79	2.05	1.88	2.00
22	1.96		1.61		1.79	1.70	1.77
24	2.23		1.79		1.88	1.79	1.92

Eruption increments in millimetres (per 48 hours), corrected for radiographic distortion.
Group 1 (Controls).

Day	BM4	BM5	BM6	BF4	BF5	BF6	Mean
2	2.05	1.70	1.96	1.61	1.61	1.96	1.81
4	2.23	1.96	1.88	1.88	1.61	1.88	1.91
6	1.88	1.88	1.79	1.70	1.61	1.88	1.79
8	2.05	2.14	2.32	2.05	2.05	2.05	2.11
10	2.05	1.88	1.88	1.79	1.88	1.96	1.91
12	1.61	1.79	1.70	1.96	1.70	1.70	1.76
14	2.23	2.05	2.32	1.88	1.70	1.70	1.98
16	1.96	1.96	2.14	1.96	1.88	1.52	1.90
18	2.32	1.88	2.05	1.88	1.88	2.05	2.01
20	2.23	1.96	2.05	1.79	1.61	1.88	1.92
22	1.70	1.70	1.96	1.70	1.70	2.05	1.80
24	2.23	1.79	2.14	2.05	2.14	1.88	2.04

Eruption increments in millimetres per 48 hours,
corrected for radiographic distortion.
Group II (Guanethidine).

Day	AM1	AM2	AM3	AF1	AF2	AF3	Mean
2	2.14	2.14	1.88	2.23	1.96	1.88	2.04
4	1.88	2.05	1.96	2.23	1.96	1.96	2.01
6	2.41	2.14	1.96	2.14	1.79	1.96	2.06
8	2.05	1.88	1.96	1.88	2.05	1.79	1.94
10	1.96	1.43	1.88	1.88	1.88	1.79	1.80
12	2.23	1.61	1.79	2.32	1.79	1.96	1.95
14	2.41	1.70	2.05	2.41	2.32	2.23	2.19
16	2.05	1.96	1.96	2.05	1.88	1.79	1.95
18	2.32	1.79	1.88	2.05	2.23	2.05	1.90
20	1.96	1.61	1.88	2.23	1.70	2.05	1.90
22	1.96	1.79	1.70	1.70	1.52	1.79	1.74
24	2.32	2.14	2.23	1.88	2.05	2.05	2.11

Eruption Increments in millimetres per 48 hours,
corrected for radiographic distortion.
Group 111 (Hydralazine).

Day	BM1	BM2	BM3	BF1	BF2	BF3	Means
2	1.96	2.14	1.96	1.79	1.70	1.88	1.91
4	1.79	1.88	2.14	1.88	1.70	1.79	1.86
6	1.79	1.96	1.70	1.79	1.70	1.96	1.82
8	2.32		1.52	1.96	1.79	1.61	1.84
10	1.79	1.96	2.05	1.79	1.61	1.70	1.82
12	1.43	1.96	1.88	1.70	1.61	1.43	1.67
14	2.32	2.32	2.41	1.88	1.96	2.05	2.16
16	1.70		1.79	1.88	1.70	1.88	1.79
18	2.23		2.14	1.79	2.05	2.14	2.07
20	1.96		1.96	2.23	2.05	2.05	2.05
22	1.79		2.14	1.61	1.70	1.79	1.81
24	2.05		2.14	1.79	1.70	2.41	2.02

Eruption increments in millimetres per 48 hours, corrected for radiographic distortion.
Group IV (Demecolcine).

Day	AM4	AM5	AM6	AF4	AF5	AF6	Means
2	1.70	1.96	1.88		1.70	1.96	1.85
4	1.88	1.96	1.96	1.79	1.96	1.96	1.92
6	2.05	2.23	2.05	1.79	1.70	1.79	1.94
8	1.88	1.88	2.05	1.70	1.70	2.14	1.89
10	1.88	1.88	1.88	1.96	1.70	1.96	1.88
12	1.88	1.96	1.52	1.43	1.88	1.52	1.70
14	1.88	1.52	1.88	1.79	1.34	1.70	1.68
16	1.34	1.07	0.54	1.07	0.71	0.63	0.89
18	0.63	1.16		0.36	0.18	0.27	0.52
20		0.36		0.36		0.18	0.30
22				0.36			
24							

Eruption increments in millimetres per 48 hours, corrected for radiographic distortion.
Group V (Triethylene Melamine).

APPENDIX 6,B.

Weights in gms.

Group I (Control). Animal.

Day	CM1	CM2	CM3	CF1	CF2	CF3
0	255	250	240	190	180	180
2	260	250	230	190	180	180
4	265	255	230	190	175	180
6	260	255	230	185	170	180
8	265	260	230	185	175	185
10	270	260	240	195	180	190
12	275	260	240	195	185	
14	280	260	240	200	185	190
16	280		240	200	190	190
18	285		250	210	190	200
20	285		250	210	195	200
22					195	200
24	295		245		195	195

Group II (Guanethidine).

Day	BM4	BM5	BM6	BF4	BF5	BF6
0	225	245	240	175	150	180
2	230	245	240	180	150	180
4	235	250	235	185	160	185
6	235	250	240	185	165	190
8	240	255	245	185	160	190
10	235	265	245	190	155	195
12	235	265	250	185	155	195
14	235	270	255	190	160	195
16	235	270	250	190	165	195
18	250	275	260	195	170	200
20	250	280	265	195	175	195
22	255	280	270	195	175	195
24	250	280	260	195	170	200

Weights in gms.

Group III. (Hydralazine)

	Animal.					
Day	AM1	AM2	AM3	AF1	AF2	AF3
0	240	225	225	185	185	170
2	245	230	230	185	185	
4	250	240	240	185	190	175
6	250	240	240	185	190	175
8	250	235	245	185	190	175
10	260	240	250	190	190	190
12	260	235	245	185	190	185
14	265	240	255	185	195	190
16	265	240	255	185	195	190
18	270	255	265	195	205	195
20	280	265	275	200	205	205
22	280	270	275	205	205	205
24	275	270	275	195	200	200

Group IV. (Demecolcine)

Day	BM1	BM2	BM3	BF1	BF2	BF3
0	250	220	230	175	185	190
2	250	230	225	175	180	190
4	255	235	225	175	185	190
6	260	235	225	180	185	185
8	270	235	235	180	185	185
10	265	230	240	185	185	190
12	270	235	240	180	185	185
14	275	230	245	185	185	190
16	275		245	180	185	185
18	280		255	190	175	190
20	285		245	195	185	200
22	295		245	195	185	200
24	290		240	195	185	200

Weights in gms.

Group V. (T.E.M.)

	Animal.					
Day	AM4	AM5	AM6	AF4	AF5	AF6
0	235	235	240	185	175	175
2	235	240	245	180	175	175
4	250	250	250	190	175	180
6	250	250	250	185	175	180
8	250	255	250	190	170	185
10	255	255	250	185	175	185
12	235	250	240	175	165	175
14	225	235	225	170	155	165
16	210	210	205	150	135	150
18	180	185		150	125	140
20		165		140		125
22				145		

APPENDIX 6,C.

Group Boundary x values	$x - \mu$	$X = \frac{(X-\mu)}{S}$	P(X)	$\Delta P(X)$	$E = \Delta P(X) \times 159$	O	O - E	$\frac{(O-E)^2}{E}$	E
1.48	-0.4333	-2.5265	.0058						
				.0169	2.69	1	-2.61	1.89	
1.57	-0.3433	-2.0017	.0227						
				.0471	7.49	6	-1.49	0.30	less than 1.57 =3.61
1.66	-0.2533	-1.4770	.0698						
				.1007	16.01	21	4.99	1.56	
1.75	-0.1633	-0.9522	.1705						
				.1640	26.08	25	-1.08	0.04	
1.84	-0.0733	-0.4274	.3345						
				.2043	32.48	33	0.52	0.01	
1.93	0.0167	0.0974	.5388						
				.1943	30.89	31	0.11	0.00	
2.02	0.1067	0.6222	.7331						
				.1412	22.45	17	-5.45	1.32	
2.11	0.1967	1.1469	.8743						
				.0784	12.47	15	2.53	0.51	
2.20	0.2867	1.6717	.9527						
				.0333	5.29	5			more than
2.29	0.3767	2.1965	.9860						
				.0108	1.72	4	2.48	0.82	2.20 =7.52
2.38	0.4667	2.7213	.9968						
				.0026	0.41	1			
2.47	0.5567	3.2461	.9994						
					159.00	159	0	6.45	

Mean = 1.9133
S.D. = 0.1715
n = 159.

Degrees of Freedom = 9 - 3 = 6.
The 5% point for chi-square with 6 degrees of freedom is 12.59.

All Controls.